Biodiversity, Biosolids and Bioindicators in *Pinus radiata* D. Don Planted Forests

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A thesis
submitted in partial fulfilment
of the requirement for the degree
of
Doctor of Philosophy
from the
University of Canterbury
by
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University of Canterbury
2003

Errata. Please replace the data in Table 3.14 (Chapter Three, page 70) with data shown below.

Table 3.14 ANOVA Summary statistics of the mean abundance of taxa in functional groups at Hunter's Road for the post-treatment survey.

Source	SS	df	MS	F value	P
Treatment	4.98	5	0.99	4.55	0.024
Residual	7.57	12	0.63		
Total	12.56	17			

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ACKNOWLEDGEMENTS

I am indebted to the School of Forestry, New Zealand *Forest Research,* Christchurch City Council, CSIRO (Canberra) and the Selwyn Plantation Board for the financial, intellectual and practical support provided during the progress of this thesis.

I would especially like to thank the suite of supervisors who have guided me with great patience, including Professor Roger Sands, Dr Eckehard Brockerhoff, Dr Peter Clinton and Dr Ken Hobson. Additional help and inspiration from Mr Peter Johns and Mr John Hutcheson has been invaluable in cementing my understanding of New Zealand entomology. I have especially valued the ideas, discussion and interpretation of the data offered by them.

In collaboration with Mr Eric Hines (CSIRO, Canberra) and histological technician Anne Prins, I was privileged to have access to advanced techniques and equipment in Australia to undertake the histology section of this thesis. This interaction was made possible by the generosity of New Zealand *Forest Research.*

I am also grateful to Mike Gilson and the technicians at the Christchurch City Council Wastewater Treatment Plant at Bromley for making available their time and analytical equipment. I thoroughly enjoyed the time I spent working on my sample analysis in their laboratory and acknowledge the cost of processing was met by the CCC.

My appreciation is also extended to the general staff at the School of Forestry. The office administrators, Victoria Mackisack and Jeanette Allen always paved the way smoothly for me. The technical staff, including Karl Schasching, Paul Fuller and Vicky Wilton, readily responded to my endless requests for equipment, space and vehicles.

To my fellow postgraduate students, I extend thanks for the companionship, support and social interaction that made my time at the School of Forestry so memorable.

ABSTRACT

The global issue of biodiversity was addressed at a local level in this thesis, which examined the effects of a biosolids application programme on the diversity of soil and litter arthropods in *Pinus radiata* D. Don planted forests in mid Canterbury, New Zealand. A taxonomic inventory of selected sites not only added to the sparse records of entomological data for similar habitats across New Zealand, but also enabled the development of a predictive model for comparison and validation in forward research. Evidence was sought for biosolids-mediated effects in the field on (i) the functional diversity of the arthropod assemblage, (ii) Shannon-Wiener diversity (H') and (iii) an ecologically relevant, invertebrate bioindicator. This novel bioindicator, the larval cranefly *Leptotarsus* spp. (Diptera: Tipulidae) was further used in a laboratory manipulation in an attempt to quantify the ecological relationship between the genus and soil physical properties. It was then subject to chronic toxicology tests toexplore the histological effects of dietary uptake of Cu and Zn. Damage to the gut tissues and a differential effect on the architecture of the fat bodies was shown by histochemistry and light micrography. This evidence providing support for the "scope for growth" hypothesis, which suggests the allocation of energy to detoxification may impact on an organism's energy reserves, thus affecting growth and development. The larval cranefly *Leptotarsus* spp. is recommended as a pliable and responsive organism for chronic metal toxicity testing.

No support was found for the general hypothesis of a biosolid-mediated effect on arthropod functional diversity at the community level or on the diversity (H') of species. However, there was unequivocal evidence of a significant negative effect on the abundance of the larval cranefly *Leptotarsus* spp. in the field where dewatered biosolids are applied at rates beyond 400 kg N/ha. It was predicted that cranefly species with a brachypterous female would most likely be affected in forests receiving biosolids applications above 400 kg N/ha. The four cranefly species identified from the study sites were the sole representatives of the myceto/geophagic functional group. Although biosolids applications are likely to constrain both the local diversity and abundance of craneflies, the species redundancy hypothesis predicts ecological processes are unlikely to be affected. Laboratory manipulations failed to show linkage between cranefly larval abundance and their contribution to the generation of porous space in the soil, although these larvae are expected to mediate other soil-related processes, such as the redistribution of fungal inoculants.

Arthropod diversity under *P. radiata* is dominated by generalist species. Greater than 50% of the arthropods trapped were indigenous. The sampled forests clearly provide a refuge within the

agricultural mosaic and contribute to the retention of local biodiversity. The trophic structure of the arthropod assemblage was dominated by predatory species. A positive relationship between species diversity (H') and the stage of development of a stand was best described by a logarithmic curve, indicating diversity (H') increased most rapidly during the first (approx.) 10 years of forest establishment. The suites of indicator species identified as significantly indicative of the *P. radiata* habitat are expected to simplify future assessment at the long-term monitoring sites by offering an investigative tool for the non-specialist.

This thesis provides information on the community structure of a highly modified habitat which is of interest to both entomologists and forest managers. As well as giving information on seasonal abundances in relation to trapping methodologies, it provides baseline data and predictive models useful for comparing long-term effects and suggests appropriate bioindicator species as tools for straightforward and cost-effective monitoring in the future. In practice, the main results demonstrated that although biosolids-mediated effects in the *Pinus* habitat were effectively quantified at the level of genus, forest managers can have a high level of confidence that a significant biosolids-mediated effect on invertebrate biodiversity at the community level is unlikely, at least in the short term, at advised rates of application.

CHAPTER ONE

GENERAL INTRODUCTION

A. BACKGROUND TO THE RESEARCH

Biodiversity, sustainability, resource management and the "triple bottom line"

The linkages between biodiversity, sustainability and resource management have emerged as key discussion points in recent years. *Biodiversity* encompasses the variety of all biological life on earth, "including the elements of the diversity of ecosystems, the diversity between species and genetic diversity within species" (Lammerts van Bueren and Blom 1997). *Sustainability* is a concept that incorporates time and infers an activity should be conducted in such a manner as to ensure and uphold the on-going availability of a resource or provision of a service (W.C.E.D 1987). *Resource management* refers to the appropriate stewardship of naturally occurring biological components and processes upon which human existence is dependent (Glasson *et al.* 1994).

Biodiversity, sustainability and resource management are inextricably linked because it is no longer socially, politically, economically or ecologically acceptable to perceive of the world's resources as infinite, nor its ecological processes dispensable. This perception has been reinforced by a number of international recommendations and agreements (W.C.E.D 1987, Anon. 1995, 2000/01c). Accordingly, at national, regional and local levels, it has become increasingly common for key land use management agencies to incorporate the "triple bottom line" as their reporting baselines (as demonstrated in, for example, Anon. 2000/01c) thereby addressing financial, ecological and social considerations in their stewardship of resources (Glasson *et al.* 1994).

The need to maintain environmental quality

There is abundant evidence that many of the ecosystems dominated by humans are both stressed and dysfunctional (Rapport *et al.* 1998b). Degraded systems have often lost their physical integrity and may be challenged in their capacity to support viable indigenous biotic populations. A common result is the substitution of the existing biota with one which is characterized by invasive species with a high tolerance of human-mediated disturbance (Yeates 1991, Bardgett *et al.* 1994, Beaudry *et al.* 1997, Andres 1999, Bengtsson *et al.* 2000). Disturbance includes both physical and chemical distortion of the existing abiotic structure which may lead to a reduction in ecosystem performance (Attiwill 1994, Naeem *et al.* 1994). Unsustainable land use practices have

contributed to a widespread reduction in present-day environmental quality. A major task now, is to acknowledge the flaws associated with past practises, ameliorate the problems associated with current ones and ensure future activities do not compromise the continued survival of either resources, ourselves, or the biota with which we share this planet (Chapin *et al.* 2000). One widespread practice is to use Environment Impact Assessment (EIA) criteria (Glasson *et al.* 1994) to develop appropriate land management strategies to minimize the risk of biotic loss, where development may impact upon a particular resource. Active planning to retain biodiversity can prempt losses that may constrain ecosystem dynamics and some of the processes important for primary productivity (Bengtsson 1998, Brussard 1998, Heneghan and Bolger 1998). Examples in the forestry context include the mimicking of natural events (Brüenig 1996), sensitive planting designs and harvesting protocols (Butterfield 1999) and the retention of biodiversity reservoirs (Humphrey *et al.* 2000).

Which diversity? Which species? Which ecosystem functions?

Although planning for the conservation of biodiversity is widely advocated, its measurement and assessment is not straightforward (Bengtsson 1998). There are taxonomic limitations and difficulties interpreting even the simplest diversity indices (Gotelli and Colwell 2001). There are inherent problems in generating legitimate, between-system comparisons and benchmarks (Oliver and Beattie 1993, New 1998). The literature also abounds with inconclusive, contradictory and controversial conclusions where researchers have tried to understand the linkages between ecosystem processes and the key species or biotic groups within a system (Lawton 1994, Johnson *et al.* 1996, Bardgett and Cook 1998, Bengtsson 1998).

Many of the arguments become cyclic, such as the prolonged debate whether the enhanced richness and diversity of species in an ecosystem improves ecosystem function (Ehrlich and Ehrlich 1981, Naeem *et al.* 1994, Jones and Lawton 1995, Tilman 1996, Grime 1997, Wardle and Lavelle 1997, Schwarz *et al.* 2000). Some theorists argue that the successive removal of species from an ecosystem will eventually reach a point at which that ecosystem can no longer perform (Ehrlich and Ehrlich 1981). Others argue that the widespread functional redundancy of species within an ecosystem will protect that system if redundant species are removed (Lawton and Brown 1994, Andren *et al.* 1995). There is, however, general agreement that it may not be possible to recover losses incurred and therefore, where biodiversity is threatened, a precautionary approach may represent the best option.

Progressing the debate

Because it remains unclear at what point a reduction in biodiversity might result in a loss of system function (Bengtsson 1998), it is difficult to provide a quantifiable endpoint to assess the effect of management actions upon biodiversity. It is therefore imperative that a scientific basis for maximizing biodiversity values in the production estate is sought, in order to provide an understanding of the ecosystem and enable the development of integrated approaches to managing for both biodiversity and production. A case in point in the context of commercial forestry is where managers are pressured to conduct operations in such a way as to minimize environmental impacts and retain the integrity of the land for future production in a marketplace where both the demand for wood and wood products and public resistance to the harvesting of natural forests have escalated (Nambiar 1996).

Biodiversity in the production estate

Activities within both pastoral and forest production estates have typically exerted gross changes to the natural structure of the landscape, its biotic components and ecological processes. The resulting ecological balance is often found wanting. For example, intervention may be required to control invasive and opportunistic weed and pest species, whilst artificial nutrients may be needed to supplement declining soil fertility (McLaughlin and Mineau 1995). The broad scale modification of the New Zealand landscape has been shown to alter the historic diversity of many species (Yeates 1991). However, it is also interesting to note that some existing landuse activities, such as plantation forestry, have originated after prior land clearance and the failure of initial land use ventures, such as agriculture. It is possible that the planted forest may offer a range of values for biodiversity. This raises interesting questions about the resilience and resistance of the biotic assemblage within the production estate and their capacity to adequately provide ecosystem services.

B. THE ISSUES ADDRESSED IN THE STUDY

Biodiversity, biosolids and bioindicators

The thesis addresses the global issue of biodiversity and reports on findings at a local level. Specifically, the thesis examines the issue of invertebrate diversity within exotic planted forests to which dewatered sewage sludge (biosolids) is applied. In this thesis, an hierarchical approach is used to address invertebrate biodiversity, and the manner in which it could be affected, at different scales, varying grain and sequential levels of organization (Schulze and Mooney 1993, Setälä *et al.* 1998, Bengtsson *et al.* 2000).

Biosolids applications have a two-fold advantage. Firstly, they add nutrients to the forest soils, thereby enhancing tree growth and productivity (Henry *et al.* 1994, Chester 2001). Secondly, they can provide a sound alternative to disposal in landfills, waterways and the ocean (Cameron *et al.* 1997). However, biosolids introduce novel physical and chemical changes to the forest floor habitat, which are correlated to both the method and rate of application, as well as the biosolids constituents (Berrow and Burridge 1980, Bourke *et al.* 1997, Bragato *et al.* 1998, Carnus 1999). Biosolids effects may include surface crusting, altered nutrient and moisture status of soils and the elevation of trace metal concentrations in both soil and litter (Anon. 1996). Such effects can pose risks and challenges to the existing soil and litter fauna associated with the forest.

Projected outcomes

The outcomes have a high level of relevance in terms of sustainability within production forestry, because management activities, which result in a reduction in the resilience of an ecosystem, reduce economic opportunity (Rapport *et al.* 1998b) compromise the vigour and stability of an ecosystem (Johnson *et al.* 1996, Harding 1999, Herrick 2000) and contribute to a reduction in organizational complexity. Ultimately the overall health of the system may be impaired (Haskell *et al.* 1992). Thus, the outcomes can be related to key theories linking biodiversity to ecosystem stability, function and structure.

In search of a scientific basis for maximizing biodiversity

The planted forest offers a great deal of scope to examine the theoretical and practical issues associated with the integration of biodiversity with ecosystem function. This is especially so where long term monitoring sites are established, thereby facilitating parallel, interdisciplinary research, which complements our understanding of processes, promoting successive research based on the outcomes generated and questions arising (Anon. 1994a, Rivas Palma 2000).

Biodiversity inventory

How much biodiversity do we actually need to maintain the effective provision of ecosystem functions and services? Which of these services may be affected by a specific management option? A first step may well be to examine how much biodiversity we actually have. In 1998 when this research was initiated, the immediate need was to survey two nominated sites and develop a taxonomic inventory of the soil and litter invertebrates associated with these sites. The survey outcomes not only provided springboards for the development of subsequent areas for my research but also provide local entomological data for specific habitats which have not previously been well-documented.

Biodiversity in a dynamic and manipulated habitat

The characterization of biodiversity in the planted forest can only provide a generalized snapshot in time, as it is progressively coloured by dynamic processes in the habitat. The ecological rules can change rapidly within any site, for example, when the physical environment is modified during establishment, silviculture and harvesting activities (Scheu and Parkinson 1995, Paquin and Coderre 1997). The chemical environment of the soil can also be expected to alter following the application of fertilizers and essential trace elements (Theodorou and Bowen 1990). The promotion and maintenance of biodiversity in the planted forest may therefore be continuously challenged as forest managers constantly seek ways to enhance the capacity of the planted forest to produce quality trees (Fife and Nambiar 1997).

The effects of chemical fertilization on planted forests are generally of a short duration, due to the relatively small amounts applied relative to the nutrient capital of the site and the capacity of the soil to immobilize the added nutrients (Prescott *et al.* 1993). Long-term improvements in site fertility have been recorded, where sewage sludge is applied as a slow release fertilizer, particularly on phosphorus-deficient soils (Brockway *et al.* 1986). A database generated by 10 years of research and demonstration in Australia, has shown that biosolids applications are a very effective and environmentally appropriate fertilizer for plantation pine, with increases in basal area of up to 30% not being uncommon (G. Kelly, pers. com.).

The ecological assessment of biosolids fertilization on biodiversity in the forest is not straightforward. Impacts upon species may vary across regions and within localities (McLaughlin and Mineau 1995) depending on site-specific forest structure, microclimate and the inherent variation in their distribution (Humphrey *et al.* 1999, Werner and Raffa 2000). Outcomes may be clouded by the natural backdrop of temporal variation expressed by many organisms (Wardle *et al.* 1999). Litter dynamics, decomposition and forest productivity may vary, according to plantation species, the method of application and the degree of integration with the soils (Henry *et al.* 1994, Luxmoore *et al.* 1999).

There is evidence of differential effects of biosolids on nutrient turnover (Prescott *et al.* 1993) and variable effects on decomposition rate and the accumulation of litter material (Magill and Aber 1998). Negative impacts of biosolids on nutrient availability and soil processes have also been reported (Harrison *et al.* 1996). The addition of nutrients may stimulate microbial and soil

arthropod biomass and activity in predictable patterns (Hasegawa and Takeda 1996) although the duration of these responses may be limited by nutrient bottlenecks (Roth 1992, Irmler 1995).

Biosolids and heavy metals

Debate abounds regarding the short and long-term consequences of biosolids applications on the biodiversity of soil organisms and metabolic processes in forest soils, given the background concentrations of heavy metals in the sludges (Chang *et al.* 1986, Cameron *et al.* 1994, Cameron *et al.* 1997). There is good evidence that heavy metals will accumulate following repeated applications of biosolids derived from urban and industrial waste (Carnus 1999). Guidelines limiting metal concentrations in agricultural soils effectively place a ceiling on the duration of a biosolids application programme (NZDH 1992, Cameron *et al.* 1997). However, this ceiling may fail to recognize the effects of chronic metal exposure on the biota and the associated consequences for local biodiversity.

Heavy metals and bioindicators

Experiments have indicated a high level of variation in the relative sensitivities of invertebrate species to specific metals and their concentrations can be expected (Sloof *et al.* 1983, Will and Suter 1994). Similarly, the species level response may vary according to behaviour (Larsen *et al.* 1994) and the physical niche occupied (Krogh and Pedersen 1997). For example, the enhanced bioavailability of metals in acidic soils may compromise the demographics of geophagic species (Hopkin *et al.* 1989, Spurgeon *et al.* 1994, Speir *et al.* 1999). For these reasons at least, there have been repeated calls for an extension of the types of organisms used in toxicity tests and in particular, the use of ecologically relevant organisms (Hopkin 1990, Rundgren and Nilsson 1997, Maltby 1999). In at-risk habitats, there is a need to establish and monitor the background levels of metals and relate this to the metal levels in relevant species to develop a realistic model of bioavailability (Davies 1992, McLaughlin *et al.* 2000).

Histological indicators

Although toxicity tests identify potentially toxic levels of effect, they alone fail to elucidate the mechanisms by which the response is mediated at a cellular level and the patterns of defence adopted by the organism to ameliorate the toxic effect. A variety of techniques have been developed to detect and map metals in the invertebrate body (Borovansky 1997). Histopathology and histochemistry remain popular means of visually and chemically identifying the distribution of specific metals in tissues (Doughtie and Ranga Rao 1984, Bedrick *et al.* 1986).

Pathways for the uptake of essential metals by invertebrates exist at the cellular level and ingested metals may be bound to proteins or stored in granules. The form and function of the granules (Brown 1982) and the sites of accumulation have been examined for several species (Sohal *et al.* 1976, Prosi and Dallinger 1988). Internally, the distribution of metals is known to follow a heterogenous pattern, which is both metal and taxon specific (Hare 1992). It is generally accepted, however, that cells intimately associated with the digestive tract are the primary sites of uptake. Chronic exposure of test organisms to toxic elements and the subsequent examination of the tissues may elucidate species-specific mechanisms, enabling estimation of their tolerance to exposure.

It is of both theoretical and practical value to ascertain the link between the physiological cost of detoxification and its effect on an individual's fitness. Various hypotheses linking the cost of detoxification or physiological stress to differential growth, reproduction or survival parameters have been examined for a limited number of bioindicators species, primarily the Collembola, isopods and earthworms (Bengtsson *et al.* 1985, Jones and Hopkin 1998). Most have agreed that the residual cost is seen in lowered demographic parameters. Ultimately, the cost at the ecosystem level may be a reduction in biodiversity through the loss of viable populations.

C. AN OUTLINE OF THE THESIS STRUCTURE

This thesis integrates the three key topics of biodiversity, biosolids and bioindicators. The arthropods associated with the soil and litter of exotic *Pinus radiata* D. Don planted forests in mid Canterbury, New Zealand provide the tool to investigate the linkage between these topics. This thesis is broad in scope and an hierarchical approach was taken to address the research questions. These questions arose from the Christchurch City Council's 1989 proposal to utilize planted forests for biosolids redistribution, for which a resource consent was granted in 1999. The effect of biosolids applications on biodiversity in these planted forests was not included in that proposal, on the premise that these planted forests lacked a significant diversity.

The main intent of this thesis was to quantify, predict and assess the relationship between representative *P. radiata* stands in mid Canterbury and arthropod biodiversity in relation to a biosolids application programme. Subsequent toxicology tests were conducted on a novel and ecologically relevant bioindicator organism. The thesis reports from both applied and theoretical perspectives at sequential scales of effect.

The research reported is topical and has a high degree of relevance for the sustainable management of resources. The research examines questions at various levels of organization (cellular, species, population, trophic level and ecosystem) in order to demonstrate a variety of responses to habitat manipulation.

The outcomes of the thesis are expected to: (i) supplement the sparsely documented and poorly understood taxonomic diversity of the invertebrate assemblage associated with *P. radiata* planted forests in New Zealand; (ii) characterize the successional development of the invertebrate assemblage through stages of the rotation; (iii) quantify the capacity of an bioindicator species to contribute to an essential ecological process; (iv) identify a suite of invertebrate species which could be suitable bioindicators of effect for future monitoring; (v) identify differential effects of tissue-level induced Cu and Zn toxicity on a selected bioindicator.

D. RESEARCH QUESTIONS AND HYPOTHESES

Biodiversity, biosolids and bioindicators are linked in this thesis by the main hypothesis that biosolids applications are a novel disturbance event which have the capacity to alter local arthropod biodiversity. This is because the physical structure and chemical components of the biosolids have the potential to mediate change in the arthropod habitat. The time scale of these changes varies, from the short to the long term. This thesis examines evidence for such effects at a range of scales and contrasting resolutions. It is contended that if biodiversity is altered to a point at which resistance and resilience of ecosystem components are permanently compromised, both soil health and forest productivity may be threatened. The sustainability of a biosolids application programme may ultimately be linked to a better understanding of biosolids-mediated effects on invertebrate diversity and the capacity of that fauna to tolerate novel abiotic parameters.

The research hypotheses and questions addressed in the thesis were as follows:

(i) The research question which addresses local biodiversity asks "how much invertebrate biodiversity is there in the mid Canterbury planted forests?" The characterization of study sites and the development of a taxonomic database is an integral first step in recognizing and understanding the biotic composition of a specific ecosystem. This step also provided a starting point for subsequent research questions and hypotheses.

(ii) The research hypothesis which addresses local biodiversity across a range of forest development stages is "that arthropod species diversity in *P. radiata* planted forests increases as the habitat becomes more complex (heterogeneous)". This hypothesis is based on the assumption that habitat complexity develops with time, which fits well with the progressive and predictable development of the planted forest through stages of the rotation. From this theoretical approach a model was developed to help understand the biotic and abiotic dynamics of this specific ecosystem.

(iii) There are three research hypothesis linking biodiversity to biosolids. The first hypothesis proposed is "that biosolids applications would alter arthropod abundance". The second hypothesis is " that biosolids applications would alter the proportional abundance of arthropods in functional groups". The third hypothesis is "that biosolids applications would alter arthropod diversity". These hypotheses were structured to independently identify the relative risk (at least in the short term), of biosolids applications to individuals, the functional structure and taxonomic diversity. Functional diversity refers to what species "do" whilst taxonomic diversity refers to representation of individual units (i.e. families, species).

(iv) Biosolids and bioindicators are linked in the research hypothesis "that the abundance of cranefly larvae will decrease under incremental biosolids applications". A field experiment examined the biology and behaviour of the larval cranefly, *Leptotarsus* spp. in response to biosolids-mediated physical disturbance to the habitat. This experiment was used to demonstrate how the individual success of a specific genus can be threatened by the biosolids application programme, thereby potentially reducing local diversity.

(v) The research hypothesis which addresses the functional relationship between an organism and its habitat was "that cranefly larvae increase the air-filled porosity of the soil". Cranefly larvae are known to tunnel into the mineral soil of the forest floor and were therefore expected to be instrumental in creating porous space. Compacted soils can limit root elongation, which has important consequences for seedling establishment, growth and ultimately, site productivity. Soil-dwelling species which engineer porous space provide a valuable ecological function (Lavelle 1997). Such species contribute to the functional diversity of the habitat.

(vi) In the final research hypothesis, selected metal components, which are known to accumulate in soils after long-term biosolids application, are linked to the physiology of the novel bioindicator. The hypothesis tested is "that the process of metal

detoxification by cranefly larvae incurs an energy cost which may be seen as a constraint on growth." A semi-quantitative analysis of changes in gut ultrastructure and histological images are used as tissue-level response to a chronically contaminated habitat to support the "scope for growth" theory.

E. RESEARCH OBJECTIVES

In order to address the questions and hypotheses posed, seven research objectives were established. These were to:

(i) establish a baseline taxonomic inventory of the soil and litter arthropod fauna associated with two recently developed monitoring sites intended for long-term monitoring of the ecological effects of a biosolids application programme.

(ii) develop a local model relating arthropod diversity to *P. radiata* habitat complexity in relation to stage of stand development.

(iii) quantify the potential risk of biosolids, to local functional diversity, species diversity and individual success.

(iv) develop a suite of arthropod bioindicators predictable for an age class and suitable for future rapid ecological assessment.

(v) evaluate the capacity of an ecologically relevant bioindicator to alter the physical structure of the soil.

(vi) quantify differential effects of chronic trace metal contamination on the survival and growth demographics of a novel bioindicator.

(vii) identify histological evidence of metal-induced alterations to the gut ultrastructure of the novel bioindicator.

F. STRUCTURE OF THE STUDIES REPORTED IN THIS THESIS

This thesis comprises a literature review, followed by five research chapters. The following summary outlines the content of these chapters, their intent and the methodology employed.

Chapter Two provides a review of the literature. This develops the key areas addressed in the Introduction and provides an overview of the current state of knowledge, the theories and the gaps in our understanding of the underlying themes in this thesis. The review links global considerations and ecological theories to local scale issues and questions. The topics presented include:

(i) Biodiversity, ecosystem function and sustainability. Key definitions are provided and the linkages, both theoretical and empirical, are examined, with particular emphasis on the sustainable management of resources.

(ii) Arthropod biodiversity in New Zealand's *Pinus radiata* planted forests. The limited published information on the taxonomic and functional status of the arthropod fauna associated with exotic softwood plantations in New Zealand is highlighted. The importance of establishing baseline survey data prior to the initiation of an intensive management programme is stressed. The role of the planted forest as a reservoir of biodiversity is examined in terms of the value gained by retaining ecosystem processes.

(iii) Chronic contamination of soils and the use of biosolids for soil amendment. The global experience of amending agricultural and forest soils is outlined in terms of the costs, benefits and ecological risks. The sustainability of biosolids applications in relation to habitat contamination by heavy metals is examined, as too are the current NZ guidelines for establishing limit levels for metals in soils. Their limitations are outlined in the context of widely reported species-level variations in metal sensitivity.

(iv) Predictive values of bioindicators, both in the field and in the laboratory is discussed. Emphasis is placed on the ecological relevance of bioindicators. The rationale behind the utilization of a novel indicator, the larval cranefly *Leptotarsus* spp. (Diptera: Tipulidae) is outlined. Special detail is given to the use of techniques which identify responses at the tissue and cellular level to metal uptake. Model pathways for uptake are outlined and an explanation of the sequestration process employed by many invertebrates to detoxify ingested and absorbed metals is provided.

(v) Christchurch City Council's "Biosolids to Forests" programme. An outline of the rational behind the programme and the requirements for progressive ecological monitoring during the programme are outlined. The expected value in terms of forest productivity, the sustainability of the programme and the potential risks of long-term metal accumulation are discussed.

Chapter Three briefly outlines the key physical and climatic features of the two long-term monitoring sites associated with The Christchurch City Council's "Biosolids to Forests" Programme. An in-depth taxonomic characterization of the arthropod soil and litter assemblage is presented from data obtained during a 14-month survey at these two sites using a selection of trapping methods to document patterns of seasonal abundance and activity. A post-biosolids

application survey at these two sites, representing only one season, using only pitfall traps, is also presented; this survey enabled comparisons of the effects of biosolids on arthropod diversity at varying application rates. It was predicted that biosolids would alter the suitability of the abiotic habitat for some invertebrates within some functional groups, thus either challenging their capacity to tolerate the biosolids effect or facilitating their survival. The widely used Shannon-Wiener diversity index and Sorenson's Similarity Index were used to provide a numeric indication of similarity between sites and within treatments. Indicator Species Analysis was used to identify suites of invertebrate species suitable for future rapid assessment of ecological effects.

Chapter Four presents data from a six-week, late summer invertebrate survey in four forest stands representing an age class gradient. Pitfall trapping and quadrat litter sampling were used to examine patterns of association between species diversity and habitat heterogeneity. The species diversity/habitat heterogeneity theory predicts a positive relationship between species diversity and habitat complexity. An underlying assumption is that habitat complexity increases with time. It was predicted that species diversity would be linearly related to stand age, such that older stands would have greater diversity than younger stands. Results are presented in tabular and graphic form using Detrended Correspondence Analysis and Indicator Species Analysis. A suite of significant indicator species characteristic of an age class are given which could be recommended for future rapid assessment of ecological effects in stands in this and similar localities.

Chapter Five documents two studies. The first is a field-based study, in which the effects of incremental biosolids applications were quantified on two successive generations of the larval cranefly *Leptotarsus* spp. A subsequent laboratory experiment attempted to quantify the capacity for larvae to generate porous space in the forest soil matrix. Two bulk densities (0.9 and 1.1 g cm $^{-3}$) and three levels of larval abundance (0, 4 and 10 individuals) were used as variables. These experiments linked the reduction in the historic diversity of invertebrate species in this locality, to a reduction in diversity within a selected genus and the potential contribution of that genus to ecological processes.

Chapter Six presents a laboratory-based study, in which the novel indicator, the larval cranefly, was exposed to chronic-level concentrations of copper or metal salt-spiked soils. The demographic parameters measured were survival and growth. Sequestration and detoxification of ingested Cu or Zn from the soil habitat was expected to impose an energy cost, which would be seen as a reduction in growth and survival. In order to evaluate the short-term plasticity of the

larvae, the environmental stressor was subsequently removed and the demographics reassessed. This experiment is placed into a broader context by comparing the response of the cranefly larvae to that of other edaphic species for which routine toxicity test responses have been published.

Chapter Seven presents a semi-quantitative analysis of the ultrastructural changes observed in the cranefly larvae following the chronic toxicity test described in the preceding chapter. A series of colour plates are used to depict key ultrastructural differences observed. This chapter provides an explanation as to how metal toxicity can affect individual success by limiting its "scope for growth".

Chapter Eight presents the general discussion and conclusions. The key themes and issues of the thesis are reiterated, as too are the current state of knowledge and relevant gaps in our understanding of these issues. The accumulated evidence resulting from the research questions and hypotheses is reviewed. The extent to which the findings support or contradict previous studies is discussed. The research questions are reviewed in order to establish the extent to which this research extends general understanding and knowledge. The limitations of the work are noted, as too are the implications and suggestions for the future management and assessment of biodiversity in the *P. radiata* habitat. Forward research possibilities for the novel bioindicator are proposed.

The Appendix provides (A) meteorological data relevant to Chapter Three; (B) prefixes used to identify invertebrates in the DCA analysis; (C) a short chapter describing the methodologies and outcomes of a preliminary experiment relevant to Chapter Six; and (D) a reprint of a preliminary report presented at a conference and subsequently published in a peer-reviewed Conference Proceedings Supplement in the Australasian Journal of Ecotoxicology.

CHAPTER TWO

A REVIEW OF THE LITERATURE

A. SUSTAINABLE PRACTICE IN THE PRODUCTION ESTATE

Economics and ecology are linked by the "triple bottom line"

The sustainable management of natural resources has been embraced at both national and international scales (W.C.E.D 1987, Anon. 1994, 1995, 1998b, 1998c). The active promotion and advocacy of standards supporting the sensitive stewardship of both natural and managed resources, with the intent of retaining a resource as an asset in perpetuity, is now widespread. Managers thus charged with the responsibility of stewardship are expected to operate within a framework which reflects the increasing demands of society for sustainable practice. Sensitive stewardship incorporates social, financial and ecological parameters, which are often referred to as the "triple bottom line". Reporting in this framework (as in, for example, Anon. 2000/01b, 2000/01c) targets a balanced endpoint, promoting financial benefits from the ecologically sustainable stewardship of natural resources.

The retention of natural ecological processes through sustainable practice

Basic to the philosophy of sustainable practice is a sound understanding of the ecosystem in question, the ecology of the habitat and the biota which supply those services (Brussard 1998). This includes an understanding of the resilience and resistance of the system to management impacts (Johnson *et al.* 1996, Harding 1999, Herrick 2000) and an awareness of factors which may contribute to a reduction in organizational complexity (Yeates *et al.* 1994) ultimately impairing the overall health of the system (Haskell *et al.* 1992).

Ecological imbalances caused by human mismanagement often result in a stressed and dysfunctional system and eventually impact upon economic performance (Kooistra 1991, Naeem *et al.* 1994, Cameron *et al.* 1997, Rapport *et al.* 1998). Planted forests represent a long-term investment that needs to be managed on a continuum, ensuring a non-declining supply of quality wood through successive rotations. Effective and sensitive stewardship includes ongoing monitoring and assessment of environmental effects so as to retain the capacity of the system to supply key ecological services (Johnson *et al.* 1996).

B. BIODIVERSITY IN THE PRODUCTION ESTATE

Biodiversity is a global issue with local ramifications

The term "biodiversity' was coined by E.O. Wilson some 20 years ago and is thus a relatively new word. Although biodiversity issues rate highly as a topic of concern in natural ecosystems, it is only in recent years that this subject has been addressed in the context of the production estate. This interest has been, in the main, promoted by the need for natural resource managers to conduct operations with due sensitivity to ecological parameters (for example, McLaughlin and Mineau 1995). The need to describe and quantify biodiversity is fundamental to the understanding of the ecological processes, services and functions promoting that diversity in an ecosystem (Giller 1996).

For the past two decades at least, a wealth of research has been generated, which has enhanced our understanding of biodiversity as an integral part of resource management in production forests and agricultural systems (Society of American Foresters 1992, Teuben and Smidt 1992, Swift and Anderson 1993, Spellerberg and Sawyer 1996, Tilman *et al.* 1996, Bengtsson *et al.* 2000, Werner and Raffa 2000). These studies, and many others, have recognized that the management of ecosystems for biodiversity is a global issue, which can only be realized if actions are decentralized to the local scale. Relevant stakeholders are thus imbued with the responsibility and capacity to carry out appropriate actions to minimize biotic loss.

Production forests are host to a diverse plant, animal, insect and fungal biota (Huhta *et al.* 1969, Butterfield 1997, 1999, Humphrey *et al.* 2000, Jukes *et al.* 2001). A general consensus is that many species associated with planted forests are widely-distributed generalists; they also highlight the point that numerous indigenous species also utilize these habitats (Norton 1998). In a recent review of biodiversity in New Zealand's planted forests, an ecological perspective highlighted the relevance of human-induced ecosystems to the maintenance of local diversity, linking this to options for plantation management (Allen *et al.* 1995).

So much biodiversity and so little is known

On a global scale, the taxonomic diversity of species is vast and largely unexplored. For example, the number of identified microbial species is believed to account for only 3-10% of the estimated total of microbes (Hawkesworth 1991). Similarly, there are gaps in our taxonomic understanding of the invertebrate fauna. These gaps may be less pronounced in the northern hemisphere, where entomological research has a long history.

The gaps in entomological knowledge are substantial in the southern hemisphere and very pronounced in New Zealand. For example, it was noted, at least 25 years ago, that less than 50% of the 20-30,000 species of insects in New Zealand had been described and only a small proportion were understood ecologically (Kuschel 1975, Watt 1982). Even frequently encountered species may present challenges to identification beyond family level.

The compilation of community assemblages and indicator species for specific habitats has proven to be one way in which ecologists can better understand species interactions, and managers can better plan to alleviate biotic loss (Humphrey *et al.* 1999). For example, the rare grass *Alexfloydia repens* is pollinated by the butterfly *Ocybadistes knightoreum* and both are known to occur in a very restricted area of commercial forest on the mid north coast of New South Wales, Australia. Managers have fenced off the grass in at-risk areas to minimize damage from grazing and vehicles (Spencer 2002).

How can biodiversity be measured?

The actual measurement of biodiversity is not straightforward (Cousins 1991, New 1998, Gotelli and Colwell 2001). This is because biodiversity encompasses a number of levels, from the community assemblage, through to the abundance of individuals within a species through to the gene. Although the biota need to be assessed uniformly, from one level, at a biologically meaningful level, taxonomic insufficiency may be limiting. The taxonomic dilemma is a particular problem for assessing invertebrate diversity (Pik *et al.* 1999). One approach which alleviates the problem is the use of Recognizable Taxonomic Units (RTU's), to group undescribed or unidentifiable morphospecies (Oliver and Beattie 1993). Collections so formed may be later identified by experts.

Biodiversity can be measured using indices which provide both qualitative and quantitative metrics that enable comparative analysis (Noss 1999, Gotelli and Colwell 2001). Although diversity indices are useful because they reduce a community or system to a single value, reduction can lead to a loss of information. Spurious interpretations may result where comparisons are made across ecosystems. A case in point is the comparison of species diversity between natural and managed systems, which has led to the current misperception that New Zealand production forests are "ecological deserts" (Allen *et al.* 1995).

Biodiversity indices rarely advance the understanding of ecological processes and species interactions. This is because they subsume all species attributes as being similar, when in fact

they are not (Cousins 1991). Indices do, however, have a role in biodiversity assessment, if used in conjunction with other analyses, to develop an overview of the pattern of species richness, abundance and evenness in an ecosystem. It has become increasingly common to direct attention away from indices and focus on function, life histories, indicator species, and assemblages (Hutcheson *et al.* 1999).

Biodiversity can also be measured from the functional viewpoint, which involves the formation and quantification of links between the variety of species and the variety of functions undertaken by those species (Jones and Lawton 1995, Johnson *et al.* 1996, Wardle and Lavelle 1997, Bengtsson 1998). Functional diversity refers to the key roles assumed by organisms, usually via their trophic status or their capacity to partake in specific processes, such as nitrogen cycling and decomposition (Zak *et al.* 1994). Where a functional allocation is made for each species present, an inventory can be used to address the broader theoretical issues of species abundance, diversity and processes in the ecosystem. However, difficulties can arise where a species is allocated to a single functional level, when that species may alter its feeding preferences according to its developmental stage (Vlug and Harrewijn 1994). Thus, a knowledge of the life histories of the species in question is integral to discriminating communities or understanding their associations with habitat attributes (Hutcheson *et al.* 1999).

The functional approach to biodiversity research has a high level of applicability to the sustainability of managed ecosystems, as it potentially enables the clarification of biotic associations and the subsequent identification of relationships between productivity and ecosystem health (Bardgett and Cook 1998). The generation of statistically valid ecological comparisons of community assemblages and processes between, for example, a set of forest sites, may be facilitated by using indicator species analysis, which links key species with specific habitats, by combining a species' relative abundance with its relative frequency of occurrence in various groups of sites (Dufrene and Legendere 1997).

As a group, the invertebrates lend themselves to biodiversity measurement. This is because they have a rich diversity, are abundant and widely distributed throughout both terrestrial and aquatic ecosystems and maintain a pivotal role in the maintenance of fundamental ecological processes (Jones *et al.* 1994, Lawton 1994, Cranston and Trueman 1997, Frouz 1999, Paoletti 1999, Gotelli and Colwell 2001).

Hypothetical models which help explain biodiversity

A key question underlying biodiversity research is whether the enhanced richness and diversity of species actually improves ecosystem function. Do more species representing more families from different orders enhance processes, such as nutrient recycling or soil aeration? Three hypothetical models have been proposed which link biodiversity to ecosystem structure, process and function:

(i) Redundancy: this model suggests that given sufficient biomass, the loss of all but one representative species from each functional group will not significantly compromise ecosystem function (Lawton 1994).

(ii) Keystone: certain "keystone" species are considered essential for ecosystem function and their decline or absence has a cascading effect on other members of the ecosystem (Shaffer 1981).

(iii) Rivet-popper: this hypothesis suggests that all species are interdependent contributors to ecosystem function and therefore greater diversity equates with increased stability through increased linkages The loss of some species can be tolerated but there is a component threshold beyond which the system in question cannot adequately function (Ehrlich and Ehrlich 1981).

Variable support has been found for each of these models, prompting extensive debate in the literature (Swift and Anderson 1993, Lawton 1994, Naeem *et al.* 1994, Andren *et al.* 1995, Jones and Lawton 1995, Tilman 1996, Grime 1997, Rusch and Oesterheld 1997, Wardle 1997). In a recent and comprehensive review, each of the theoretical models noted above were analyzed to estimate the level of species richness required to maintain ecosystem function (Schwarz *et al.* 2000). The conclusion was that within a single trophic level, saturation of ecosystem function was predicted at a low proportion of species richness, thereby supporting the basic hypothesis of functional redundancy. This finding has interesting implications for invertebrate biodiversity in the planted forest. Is functional redundancy a feature of the assemblage in these extensively modified systems?

The value of taxonomic inventories

An essential first step in biodiversity assessment, is the compilation and identification of the species present (Miller and Lanou 1995, Scholes and Nowicki 1998). Through the documentation of the spatial distribution of biological components, inventories facilitate conservation planning, the assessment of sustainable use of natural resources and provide a basis for the selection of indicator species and assemblages (Kremen *et al.* 1992).

C. BIODIVERSITY AND THE FORESTRY CYCLE IN NEW ZEALAND'S PLANTED FORESTS

Changing perceptions of biodiversity conservation in planted forests

The ecological value of exotic planted forests in New Zealand had traditionally been perceived as relatively low (Allen *et al.* 1995). The floral and faunal assemblage is poorly understood and the research undertaken has emphasized their low biodiversity, because sites were evaluated in comparison with those supporting generally undisturbed, indigenous vegetation (McColl 1974b, Anderson and Death 2000).

In past years, ecological factors within production forests have been viewed as constraints to productivity (Whyte 1988). Nowadays, it is increasingly common to hear calls for enlightened forest management, for which managers need to be "...cognizant of the biological significance of the forests, both managed and natural..." (Noss 1999). Resource managers are now required to address sustainable practices within their domains, by facilitating the maintenance of fundamental ecological processes, monitoring the ecological continuity of functionally important species and protecting biological diversity (DoC/MfE 2000). In New Zealand, the impacts of land management on biodiversity may be an important consideration for the granting of future resource consents (Allen *et al.* 1995).

Factors affecting invertebrate biodiversity in planted forests

P. radiata is often grown on marginal lands that have previously been rejected for agricultural use and often abandoned (Will 1985). Given this background, a restricted biotic diversity is expected, because such sites generally lack the physical structure, organic resources and founder populations necessary for the establishment and maintenance of indigenous invertebrate communities with a close interdependence. It is more likely that the species present will be adventives with generalist resource requirements.

Conifer plantations, like many other forests, tend to acidify soils (Will 1985, Yeates 1989, Rosoman 1994, Scholes and Nowicki 1998, Butterfield 1999). Soils under *P. radiata* have been shown to acidify with time as the plant-available phosphorus increases, as too does the amount of carbon present in relation to nitrogen (Yeates *et al.* 2000). This effect can restrict the suitability of the habitat for some species, for example, earthworms (Lee 1985, Carcamo *et al.* 1998).

Previous studies in New Zealand have shown invertebrate diversity to be a sensitive indicator of habitat modification in relation to exotic planted forests. For example, a trial established in a

grazed pasture at Tikitere in 1973, reported that at high stocking rates (200 and 400 stems/ha), enchytraeids and earthworms both declined in abundance and the functional composition of the nematode community altered (Yeates *et al.* 2000). Similarly, the conversion of pastures and high country tussock grasslands to *P. radiata* resulted in a reduction in the diversity of nematode species present, which was possibly due to slower carbon and nutrient cycling under the exotic forests (Yeates and Saggar 1998).

In combination, historical, physical and chemical factors have clearly acted to limit biodiversity in the planted forest ecosystem. It is of both theoretical and applied interest then, to examine the composition of the resulting biotic assemblage, in the context of diversity and ecosystem function. An understanding of how resilient and resistant the biotic system is to physical and chemical disturbance, may help minimize future losses.

How much is known of arthropod diversity in New Zealand's planted forests?

From the few faunal surveys undertaken in New Zealand's planted forests, on the West Coast (McColl 1974b), in North Canterbury (Johns *et al.* 1980) and the North Island (Styles 1967, Hutcheson and Jones 1999), the consensus is of a distinctive, yet limited invertebrate assemblage. McColl (1974b) sampled the forest floor of a young *P. radiata* forest established on cut-over and burnt beech forest and noted invertebrates were poorly represented or absent in the samples in comparison with nearby beech forests. McColl recorded invertebrate abundance at ordinal and family levels and found a predominance of larval Lepidoptera and Diptera, as well as Diplopoda, Chilopoda and Araneida.

Styles (1967) gave a short summary of the early studies of the fauna associated with the breakdown of litter in both indigenous and exotic forests in New Zealand. He provided a short list of 17 identified species, as well as a summary of specimens (at the family level) extracted from pine litter over a four-year period. The microfaunal community associated with the needle litter was dominated by Collembola, omnivorous Oribatei, dipterous and lepidopterous larvae, staphylinid and trichopterygid beetles, plus predatory gamasid and trombidiid mites.

An invertebrate survey of old forests at Hanmer, North Canterbury, stocked with *Pinus nigra* var. austriaca Arnold and *P. radiata*, growing on shallow soils "...overlain by litter derived almost entirely from the needles of the respective trees..." were sampled by pitfall traps and litter quadrats (Johns *et al.* 1980). In the comprehensive species list provided, biological notes

indicated that many of the invertebrates found also enjoyed a widespread distribution across the Canterbury plains and on Banks Peninsula.

A high proportion of the invertebrates listed in Johns et al (1980) featured recognized affiliations with human habitation or modified habitats, including *Forficula auricularia* Linnaeus, 1758 (the common golden earwig), *Pleioplectron simplex* Hutton 1897 (a jumping weta common in scrub, plantations and indigenous forest), *Hypharpax* spp. (a small, black carabid commonly associated with open areas), *Creophilus oculatus* (a staphylinid commonly associated with carrion), *Costelytra zealandica* White, 1846 (a beetle pasture pest in the larval stage), *Enarsus* spp. (a small subcortical-dwelling colydiid beetle common on dead timber), *Selenopalpus aciphyllae* Broun, 1886 (an oedomerid restricted to the eastern side of the South Island from southern Marlborough downwards) and *Zelanion morbosus* Hutton 1877 (a widespread centipede species found under the bark of fallen logs). Introduced, species present in the Hanmer plantations included *Apion ulicis* (a weevil used for the bio-control gorse), *Hylastes ater* (a common pest of pine forests), *Cylindroiulus britannicus* Verhoeff, 1891 and *Ophyiulus pilosus* Newport, 1843 (two millipedes commonly associated with exotic vegetation)

Hutcheson and Jones (1999) adopted a pragmatic approach to assessing invertebrate diversity in planted forests. They used malaise traps to sample the beetle assemblages from second rotation *P. radiata* stands in Kaingaroa forest, expecting the four-week sampling effort in mid-summer to effectively account for the majority of species which are actively utilizing the forest. They found cluster analysis effectively discriminated between the age of a stand and the beetle assemblage present. There was evidence of successional processes, in which the relative abundance of component species changed according to the stage of stand development. They noted a predominance of detrivores across all traps, suggesting the recycling of cellulose (derived from pruning and thinning) may drive the diversity of the beetle assemblage in the fast growing exotic pine system.

An unpublished report (Macfarlane *et al.* 1999) of the McLeans Island area, on the south bank of the Waimakariri River, west of Christchurch, includes a taxonomic list of invertebrates associated with pine plantations and shelterbelts. The unimproved pasture areas are lightly stocked and the forested areas have been planted on the marginal rocky soils of an old riverbed. Species included *P. simplex*, *Bobilla* spp. (a small black cricket), *Lycosa hilaris* (the wolf spider), *Phalangium opilio* (harvestman), *Steatoda lepida* (a comb-footed spider), *Taieria erebus* and *Lepthyphantes tenuis* (small spiders common in wooded and suburban sites), *Metaglymma tibiale* (a small black

carabid). (It is possible that this latter carabid, generally found in Otago and Southland may have been misidentified and is more likely to be *Metaglymma moniliferum*). *Porcellio scaber* (the common woodlouse), millipedes and staphylinid beetles were also accounted for. This survey suggested the general structure of the invertebrate community in these habitats consisted primarily of vagrant and cosmopolitan opportunists dominated by predatory groups.

The way in which silvicultural practices increase the structural complexity of the forest floor has been suggested as one way in which arachnid diversity may be increased in *P. radiata* planted forests (Anderson and Death 2000). In their study, cluster analysis was used to compare spider diversity between similar-aged *P. radiata* stands in the North Island, New Zealand, which had a variable proximity to native forest and pasture. They reported that a similar community structure occurred at each of three sites.

Overall, the research to date has provided valuable taxonomic and abundance data on the invertebrate assemblage associated with *P. radiata.* However, not one of these studies have been in a position to monitor community change in relation to stand management.

Biodiversity and the dynamic planted forest habitat

The stage of a forest within a rotation (the period between planting and harvest) is a strong determinant of the demands tree growth will place on soil nutrients (Lewis and Ferguson 1993). Economic considerations drive the length of rotation (on average in New Zealand, this is 25 years) and most sites are re-established soon after harvest, often incurring substantial and intensive habitat disturbance. Mechanical harvesting and post harvest site preparation, (particularly the windrowing of harvest detritus), as well as waste thinning and pruning of younger stands, weed and grass slashing and the use of herbicides, all constitute gross habitat disturbances. Local biodiversity may be seriously reduced as a result (Bird *et al.* 2000) and the invertebrate community could reasonably be expected to be depauperate under harsh or unpredictable abiotic conditions (Brown 1988).

In Canterbury's planted forests, low levels of organic matter overlay light alluvial soil types (D.S.I.R. 1968). Nutrient losses from harvesting have been shown to amount to 5% of total N and 26% of available P, whilst windrowing increased this loss to 40% and 60% respectively (Balneaves and Dyck 1992). In second rotation forests, it is likely that nutrient limitation may restrict tree growth; for this reason, new pine plantings are often fertilized within the first six months of establishment (Lewis and Ferguson 1993). New plantings may otherwise be entirely

dependent on decomposition products provided by the soil biota. In later years (i.e. after canopy closure), the annual nutrient requirements of *P. radiata* are relatively low and only ameliorative fertilizer applications are applied to sites where there are known to be nutrient deficiencies (Will 1985). Thus, in most stands, the nutrient cycles are not grossly altered by the need for heavy applications of inorganic fertilizer in order to drive growth to an upper limit.

Planted forests are a dynamic habitat. Overseas studies have highlighted the relationship between biodiversity and the physical structure of a habitat, particularly for coleopterans and ground-dwelling arthropods (Greenberg and Thomas 1995, Greenberg and McGrane 1996). Tree growth is a strong determinant of the structure and composition of the under storey, which provides a resource for herbivores and pollinators, until canopy formation and closure limit incoming radiation and restrict its' growth (Schipper 1996). The forest floor itself is part of the dynamic process, as successive periods of needle fall lead to the accumulation and developing complexity of the litter layer. Evidence from loblolly pine (*Pinus taeda*) stands in Texas, USA suggest there is a positive association between arthropod diversity and the development of the litter layer (Bird *et al.* 2000).

Biodiversity and spatial heterogeneity

There is common consensus that the structural diversity (floristics, litter and woody detritus) of the forest floor is an important determinant of invertebrate diversity (Schipper 1996, Jukes *et al.* 2001). The spatial heterogeneity theory predicts a positive relationship between habitat complexity and species diversity (Davidowitz and Rosenzweig 1998). Thus, as a habitat increases in the type, variety and abundance of structural and functional niches available to species, so too does the diversity of species.

Arthropod-centred studies have been a popular tool to investigate and find support for this relationship. Examples include orb-weaving spiders (McNett and Rypstra 2000), small insects and arachnids (Dennis *et al.* 1998) and litter macroarthropods (Hansen 2000). At the microscale level, the variety and number of pore sizes in litter is known to be important in determining the vertical distribution of mesofauna in forest litter (Teuben and Smidt 1992).

How well does the invertebrate fauna associated with the dynamic planted forest habitat support this theory? This question is of both theoretical interest and practical importance. The theoretical model enables predictions to be generated and the observed effects of management options can be calibrated. Furthermore, an understanding of the diversity/habitat heterogeneity relationship could

identify specific periods of sensitivity or capacity for change in the diversity of the invertebrate assemblage. This could be an important way in which biodiversity is managed for where silviculture options include, for example, periodic applications of biosolids.

D. BIOSOLIDS: A SLOW RELEASE FERTILIZER USED TO ENHANCE FOREST PRODUCTIVITY

What are biosolids?

Biosolids are the stabilized organic components of municipal sewage sludge separated from effluent during primary and secondary treatment stages. This solid component is of industrial and domestic origin, consisting of organic material, grit and sand, microorganisms and trace metals. The composition and characteristics of biosolids exhibit substantial variability according to source and treatment regimes (Aarne 1979). The term "biosolids" was originally coined in the USA in 1990, to describe sewage sludge which is suitable for reuse (CIWEM 1995) in order to "...improve its image and gain public acceptance for its use as an organic fertilizer..." (Carnus and Wang 1997). Biosolids may be viewed as a pollutant or a resource, depending on the perceived value of the material and the ability of the receiving environment to utilize it.

In recent years, an interest in New Zealand in the land application of municipal wastes (both effluent and biosolids) has developed due to (i) the phasing-out of ocean outfalls and natural waterways for sewage disposal (ii) the need to conserve water and nutrient resources (iii) the high cost of landfills and incineration and (iv) the consideration of indigenous cultural values which require human waste to be returned to the land (Cameron *et al.* 1997, Carnus and Wang 1997).

Why apply biosolids to planted forests?

The land application of biosolids and organic wastes to arable cropping systems and plantation forests is widespread in the USA, UK and France and has been shown to enhance soil fertility (Cameron *et al.* 1994, Smith 1996, Carnus 1999). Forest soils are often nutrient-deficient and their enhancement through the addition of nitrogen-rich biosolids is one means of increasing the production of wood fibre whilst providing a location for the redistribution of municipal waste (Will 1985, Henry *et al.* 1994, Henry and Cole 1997, Luxmoore *et al.* 1999, Chester 2001).

Biosolids are generally used as a slow release fertilizer. Biosolids applications may prove invaluable in planted forests established on light soils or marginal lands, or where the early establishment of young trees is being sought for successive rotations. This is because productivity declines during the second and subsequent rotations of *P. radiata* have been reported (Lewis and

Harding 1963, Whyte 1973, Boardman 1978) and attributed to a range of factors, including loss of organic matter and nutrients, soil compaction and weed competition (Lewis and Harding 1963, Flinn *et al.* 1979, Sands *et al.* 1979). The general responses of forest ecosystems to biosolids applications can be predicted from models, in combination with the experience gained from existing programmes (Cole 1977, Carnus 1999). For example, Luxmoore *et al.* (1999) used a computer simulation of forest growth to model the effects of adding organic matter and N to the humus pools of Douglas fir (*Pseudotsuga menziesii*) and loblolly pine (*Pinus taeda* L.). In a 100-year simulation with increasing biosolids application rates, they reported a highly significant increase in net primary productivity of Douglas fir plantations (Luxmoore *et al.* 1999).

Land application is generally believed to be an ecologically sound method of disposal and nutrient supplementation, especially where guidelines and management regimes are appropriate for local and regional conditions. Forest-based applications generally limit the risk of pathogens infecting humans, due to the food-chain separation and the ease of excluding the public from forest blocks.

Biosolids reuse is novel in New Zealand

Whilst positive benefits of biosolids applications to forest productivity have been recorded overseas, data for New Zealand to date is generally sparse, with the exception of some preliminary information from the Rabbit Island trial near Nelson (Gielen 1999). That trial commenced in 1997 on 8-year-old *P. radiata* grown on sandy soil, to which the application of biosolids was reported to have increased tree diameter at breast height, basal area, live volume and mean annual increment in volume. The live volume in both the standard (300 kg N/ha) and high (600 kg N/ha) treatment was 35% larger than in the control treatment. The Rabbit Island trial also demonstrated that 2 years after application, there remained significant differences between control and high treatments for most nutrients and metals in the litter layer.

Biosolids as a disturbance event: what are the risks to the biota?

Biosolids applications also represent an additional disturbance (beyond normal stand maintenance and forest operations) to both the physical and chemical parameters of the forest floor. There is a clear consensus from the literature that the soil biota are demographically sensitive to biosolids amendment.

Biosolids contain organic materials which may supplement food-limited species of fungivorous arthropods in the detrital food web of forest litter. Experimental evidence has indicated that both

mite and collembolan densities increase in food-enhanced plots (Chen and Wise 1997). Evidence from a study in European coniferous forests pointed to alterations in the structure and abundance of fungal communities following organic fertilization (Hogervorst *et al.* 1993). This outcome may have been due to the elevated nitrogen levels affecting microbial populations, or alternatively, secondary effects, such as changes in soil chemistry (Scholes and Nowicki 1998).

Other European research has examined invertebrate-level responses to sludge applications to arable pastures and old fields. Plots receiving sewage sludge were found to have a more diverse carabid community than untreated controls (Larsen *et al.* 1996). The annual emergence abundances of Diptera was shown to be higher in sewage sludge fertilized fields than alpine grasslands (Hoevemeyer 1999). Species-level changes in eudaphic collembola have been noted in sludged plots in field trials (Bruce *et al.* 1999). Cole *et al.* (2001) combined pitfall trapping with suction sampling to monitor epigeal/hemiedaphic Collembola in sludge-amended plots in Scotland and clearly identified the response to be species-specific, with some species actually being favoured by the application of metal-rich sludge.

Chronic contamination of soils following biosolids applications

The topic of sewage sludge and the potential problems incurring from its use in the agricultural context surfaced in the literature during the 1960's (Le Riche 1968). In that study, attention was drawn to the appearance of zinc-induced chlorosis in crop plants in a market garden experiment where sewage sludge had been used to manure soils for many years. In the ensuing years, the costs and benefits of sewage sludge, or biosolids, as an agricultural fertilizer, have been examined widely, often in the context of heavy metal contamination (Davies 1992).

Guidelines to limit biosolids-associated risks to the biota (and humans)

Guidelines for the safe use of sewage effluent and biosolids on land are provided by the New Zealand Department of Health (NZDH 1992) and are based broadly on European Guidelines (CEC 1986). They are significantly more conservative than United States Environmental Protection Agency (USEPA) guidelines (McLaughlin *et al.* 2000). The New Zealand biosolids guidelines primarily protect aspects of public health, placing reliance on the protection of soil quality on guidelines developed by the Australia and New Zealand Environment and Conservation Council and the National Health and Medical Research Council (ANZECC/NHMRC 1992). Limits have been set for the maximum permitted concentration (MPC's) of metals in biosolids, yet limits for the maximum cumulative loading rates (MCL's) and maximum annual loading rates (MAL's) for soils receiving biosolids are without a time

frame. This can have substantial consequences in terms of chronic accumulation of metals in the soil.

Although clear parameters for the application of sewage sludge to land in New Zealand are generally lacking, the one encompassing intention of national guidelines (in the case of heavy metals) is to prevent the accumulation of metals in the food chain and limit toxic effects on the plant and soil fauna (McLaughlin *et al.* 2000). Thus, any activity utilizing biosolids should, ideally, be addressing the soil, its' biota, and soil processes, in relation to the sustainability of a biosolids application programme.

Regulatory frameworks for soil contaminants

A recent review of the regulatory framework for metalloid contaminants in agricultural land in Australia and New Zealand (McLaughlin *et al.* 2000) highlights the integrative role assumed by the RMA (1991) and the delegation of responsibility for the development of policies and plans to the level of local governments and regional councils. The RMA (1991) does not provide statutory threshold levels for waste applications. It instead focuses on "the effects of activities, rather than the activities *per se* and is designed to minimize adverse effects on the environment" (Roberts *et al.* 1996).

Authorities in Australia and New Zealand have moved to protect the environment from metalloid contamination through the regulation of disposal processes and the development of guidelines in order to encourage environmentally sound practice (NZDH 1992). It is largely due to the paucity of ecologically relevant local data that many of these regulations and guidelines are based on data from overseas (McLaughlin *et al.* 2000).

The identification and testing of indicator species which are ecologically relevant in the local situation has identifiable benefits for managers and authorities. It increases the number of test species for which formal data has been recorded; it increases the confidence with which decision can be made; it addresses a global issue at a local level and; it extends our understanding of invertebrate physiology.

E. THE CHRISTCHURCH CITY COUNCIL'S "BIOSOLIDS TO FORESTS PROGRAMME"

Biosolids reuse can solve several problems at once

The land-application of biosolids has been identified by the Christchurch City Council (CCC) to have environmental, practical and economic benefits. The application of biosolids to plantation

forests is a relatively novel activity in New Zealand, although programmes utilizing sludge in Nelson forests and municipal effluent in Rotorua forests have been developed (Bourke *et al.* 1997).

Since May 1989, the CCC has actively developed the "Biosolids to Forests" programme for the reuse of biosolids on the privately owned Selwyn Plantation Board Limited (SPBL) *Pinus radiata* plantations in mid-Canterbury. A trial application programme commenced in late 2000. The biosolids, applied by a mechanical spreader, is in the form of a dewatered cake (25-30% solids), which substantially lowers both volume and transport costs (Outwater 1994).

The resource consent granting process

In New Zealand, current legislation (New Zealand Resource Management Act (RMA) 1991) dictates strict criteria for the land application of biosolids on agricultural land. The resource consent granted to CCC in 1999 permits applications of dewatered biosolids at a rate of 400 kg N/ha every second year to selected plantations. The composition of the biosolids and New Zealand Department of Health (NZDH) maximum permitted concentrations (MPC) for metals in arable soils are shown in Table 2.1. The New Zealand Water and Wastes Association has suggested MPC's may be higher in soils used for activities other than agriculture (McLaughlin *et al.* 2000*)*

Table 2.1 Composition of dewatered biosolids from the Christchurch Wastewater Treatment Plant and New Zealand Department of Health (NZDH) maximum permitted concentration (MPC) of metals in soils for agricultural use.

Biosolids components	Mean composition of biosolids [#] (mg/kg)	NZDH MPC for arable land* (mg/kg)
Nitrogen	4.0%	
Phosphorus	1.5%	
Potassium	0.25%	
Arsenic	13.8	10
Cadmium	10	3
Chromium	1667	600
Copper	462	140
Lead	266	300
Mercury	5	1
Nickel	100	35
Zinc	1519	300

Source:[#] (Anon. 1996) * (McLaughlin *et al.* 2000)

As part of the consent-granting process, CCC was obliged to develop a comprehensive monitoring and management plan for the assessment of the ecological effects of their "Biosolids to Forests" programme (Anon. 1996, 1998a). In this respect, "ecological effects" refers to aspects of the programme pertaining to operational impacts, soil nutrient and heavy metal leaching,

groundwater quality, risks to surface water, odour impacts on surrounding property owners, as well as dust and the aerosol dispersal of bacteria.

Preliminary findings provide guidelines

Background data obtained from a preliminary one year CCC trial conducted during 1992/93 at Eyrewell and Chaney's Forests (north of Christchurch), where the sludge was applied as a slurry, provided estimates of local impacts, and was utilized extensively in the formulation of the assessment plan (Anon. 1994a). Key conclusions from that preliminary study were that (i) the selection of an application rate was dependent on the ability of the forest system to store and utilize nitrogen; (ii) the annual loading rates between 200 and 400 kg N/ha were appropriate for similar forests; (iii) the retention of heavy metals in the surface soils limited the duration of an application programme; and (iv) there were advantages in applying biosolids as a dewatered cake rather than as a slurry.

The assessment plan discounts biodiversity

Although the resulting assessment plan clearly addressed the pertinent *abiotic* parameters of concern, it summarily dismissed *biotic* aspects, noting "...ecosystems within pine plantations are lacking in diversity (and) at the proposed application rates, livestock, plants or wildlife are unlikely to be adversely affected..." (Anon. 1996). If it is understood that the invertebrate soil, litter and ground fauna, although not directly addressed, are included under the broad umbrella of this statement, then several important issues are raised:

(i) the definition of *diversity* has been applied in its most simplistic form, referring essentially to indigenous, rare and threatened species;

(ii) that a narrow definition of diversity ignores the contribution of adventive, generalist and cosmopolitan species to ecological processes in the forests;

(iii) the invertebrate soil, litter and ground community associated with NZ pine plantations and their trophic relationships are not well-known, thus their responses (short or long-term) to the physical and chemical effects of biosolids cannot be gauged;

(iv) irrespective of the proposed biosolids application rates, the introduction of a nutrient-rich material with a trace metal content to a typically nutrient-deficient ecosystem can be expected to have both short and long-term effects on the composition of the forest floor invertebrate community; and

(v) the lack of background information on the probable effects, indicates that the likelihood of *adverse effects* is difficult to estimate.

For these reasons, the management plan fails to address a key tenet of biodiversity, which is to preserve the ecological integrity of systems through the protection of the fauna contributing to ecosystem function. As cultivated systems, including forestry, are reliant on these functions (i.e. decomposition and nutrient recycling) for yield stability, their conservation may have important economic repercussions.

This thesis contends that the proposed ecological monitoring programme for the CCC "Biosolids to Forests" programme has inadequately addressed the issue of biodiversity. The sustainability of the programme is limited by the accumulation of soil metals, which is recognized to be a poor indictor of the bioavailable fraction (Crommentuijn *et al.* 1997, Peijnenburg *et al.* 1999). There is a need to more fully understand the structure and composition of the biotic community most at risk from an application programme; there is a need to identify potential species of value as indicators for future rapid assessment; and there is a need to recognize the integration of the planted forest within the local district as a valuable resource for the maintenance and enhancement of local biodiversity.

F. BIOSOLIDS AND CHRONIC METAL CONTAMINATION OF THE SOIL AND LITTER

Biosolids: there is no such thing as a "free lunch"

The long-term beneficial properties of biosolids applications to forest soils are limited by the accumulation of trace metals and organic micropollutants (Chang *et al.* 1986). One of the most important conceptual differences between biosolids *applications* and biosolids *disposal* is that "....the soil and vegetation cover must be beneficiaries of the application and not simply recipients of the material...." (Cole 1977). In the case of the Christchurch City Council's programme, the biosolids application programme is perceived as a "win-win" situation, as the "free" nutrient-rich biosolids are expected to enhance productivity (of benefit for the forest owner) and also solve the council's problem of sewage disposal.

However, there are a variety of risks associated with biosolids applications. Managers of plantation forests receiving biosolids need to consider the trade-offs between the short-term benefits (e.g. enhanced growth through improved availability of nutrients) and the chronic and long-term effects (e.g. heavy metal accumulation in litter and soils), because biosolids applications may be detrimental to ecosystem functioning, thus limiting future land-use options (Sauerbeck 1987, Bruce *et al.* 1999).

Metals of concern in the Christchurch biosolids

The industrial metal contaminants discharged into the Christchurch wastewater stream are primarily lead, cadmium and chromium, which are derived from meat and dairy processing, tannery wastes, the textile and beverage industries, wood processing and basic metal industries. The domestic input of metals is not insignificant and includes copper, zinc, lead and nickel. Typical sources include the corrosion of metal plumbing fittings, cosmetics, health-care and household products.

The bioavailability of metals in soils

A key factor determining the reactivity, availability, uptake parameters and ultimately the toxicity of trace metals to the soil biota is metal ion speciation (Hayes and Traina 1998) which is mediated by substrate moisture content, pH and the organic content of the soil (van Straalen and Bergema 1995, Crommentuijn *et al.* 1997). In addition, an increase in the stability of complexation interactions of metals with humic substances has been reported with increasing pH (Ghosh and Banerjee 1997). The addition of a pollutant to soils initiates changes to the structure of the soil community, initially to microbial populations, with flow-on effects to higher trophic levels. These effects are largely determined by the bioavailability of the contaminant to specific trophic groups.

As an example, springtails (Collembola) which occupy an important trophic level as regulators of decomposition (Seastedt 1984) and prey species (Hopkin 1997), are adversely affected by sludge application to agricultural land, with species-specific sensitivities varying according to levels of contaminants (Cole *et al.* 2001). The subsequent alteration to the community structure observed in that study was suggested to have implications for decomposition, soil fertility and the abundance of polyphagous predators.

A simplistic estimate of the metal content in a soil type may have little bearing on its availability to the resident biota (Rogers 1996, Hayes and Traina 1998, Peijnenburg *et al.* 1999). The corollary to this argument is that metal concentrations in the biota may only partially reflect environmental pollution levels, as factors including the age, weight and behaviour of a species can influence quantitative relationships (Dallinger 1994, Spurgeon and Hopkin 1996). Chronic effects are likely to occur under successive biosolids applications to forest soils. In a study involving the application of high rates (300 Mg/ha) of low C:N ratio biosolids to Douglas fir and grand fir (*Abies grandis*), the excess organic N mineralized, nitrified and contributed to soil acidification (Harrison *et al.* 1996). Evidence derived from laboratory-based trials indicates

elevated ecological risks to the soil biota from metals under soil acidification. For example, in soils with total lead concentrations of 85 μg/g, the ecological risk (Will and Suter 1994) to the soil biota increased from 1.5% (pH 6) to 77% (pH 3.5) (van Straalen and Bergema 1995).

Experience indicates both nutrients and metals become intimately associated with forest litter (Cameron *et al.* 1997) where arthropod biomass is highest (Huhta *et al.* 1969, McColl 1974a, Hoekstra *et al.* 1995, Beaudry *et al.* 1997). Substantial alterations to the chemical composition of the soil and litter at Eyrewell Forest in North Canterbury were reported following slurry application trials during 1992-93 (McLaren *et al.* 1994). Those trials convincingly demonstrated the strong retention of heavy metals in the top 20 cm of the litter/soil horizons under 12 and 23 year old trees, where quarterly applications were made during a 12 month period (at rates of 200 and 400 kg N/ha).

The sensitivity and resilience of forest floor invertebrates to the direct or indirect effects of physical and chemical disturbance is highly variable and dependent on factors including trophic status, mode of exposure, behaviour and seasonal activity levels. It is important to consider the effects of intensive management options on these organisms as they are (in association with microbial populations) instrumental in nutrient recycling in the forest (Andren *et al.* 1995) and ultimately, forest health (McLaughlin *et al.* 2000).

The benefits to the biota of nutrient enhancement of the forest floor

There is some evidence that the effects of disturbance on the biota in planted forests may be neither negative nor permanent. For example, in a study in the USA, Bird *et al.* (2000) reported that despite substantial habitat disturbance in loblolly pine plantations (including low and high intensity harvesting, herbicides and fertilization applications), there was a rapid recovery in the diversity of the soil and litter arthropod community. Where higher arthropod species richness was recorded following fertilization treatments, it was suggested that a fraction of the invertebrate community were better able to utilize the nitrogen and phosphorus inputs. An indirect response to fertilization possibly occurred via increases in microbial biomass.

Fertilization treatments may also stimulate the development of vegetative cover, which provides shelter, food resources and a more equitable microclimate for some invertebrate species An increase in the complexity (variety) of resources in a habitat has been proposed as one mechanism by which species richness may increase.

Pulse-effects on invertebrate populations, following biosolids amendments, have been reported. In a Spanish study (Andres 1999), the addition of sewage sludge to quarry soils as part of a restoration project resulted in an increase in mean annual arthropod density. At the highest rates of application, community structure became impoverished. Populations negatively affected included some myriapods (Diplopoda: Julidae) and large predators of the mite family Parasitidae (Mesostigmata); groups dependent on organic matter, such as immature coleopterans and uropodid mites were stimulated, as too were the moisture-dependent Collembola.

Not all biota are likely to respond equally

Species-specific responses by invertebrates to metal contaminants in sewage sludge have been identified. In a field experiment in Scotland using Collembola as the test species, it was reported that the addition of uncontaminated sludge increased the abundance of *Heteromurus nitidis* and *Isotoma notabilis*, whilst the addition of cadmium-rich sludge reduced the abundance of *Lepidocyrtus cyaneus* and *Isotoma viridis* (Cole *et al.* 2001). The implications of that study relates primarily to the relative sensitivities and tolerances of different species. The soil concentration of cadmium in the cadmium-rich sludge plots was below that found to adversely affect Collembola in laboratory trials; it was suggested that adverse effects at the species level could alter decomposition rates, soil fertility and community structure.

Similar alterations in invertebrate community structure have been identified in a New Zealand study, where pasture soils were contaminated with surface runoff from timber preservative. Biological activity in the soils was suppressed at Cu, Cr and As concentrations of 400 mg/kg and inhibited at 800 mg/kg. Subsequently, a significant decline in the numbers of plant-associated nematodes (used as bioindicators of effect) was found with increasing levels of contamination (Yeates *et al.* 1994). In a parallel study, plant-feeding nematodes were identified as the predominant guild where contamination levels were low, whilst bacterial-feeding and predatory nematodes dominated the heavily contaminated soils (Bardgett *et al.* 1994).

Balancing the biotic loss with economic gain

A change in invertebrate community structure may not necessarily be deleterious to the system. Of greater interest is the resilience of the system in question and the relative plasticity of species and groups under the disturbance regime. Although it is widely acknowledged that metal contaminants adversely affect environmental parameters, the level of 'acceptable ecological damage' resulting from the chronic accumulation of metals is difficult to assess, when weighed

against economic criteria. This is largely due to the lack of a theoretical framework for the prediction and monitoring of damage (Hopkin 1990).

Seeking new ways to protect more species

Given the high level of variability of responses within and between species (Tomlin 1992, van Straalen and Bergema 1995, Wilczek and Migula 1996, Gräff *et al.* 1997), future progress in the effective risk assessment (Leeuwen 1990) and management of chronically contaminated habitats (and those with the potential to become chronically contaminated) will be dependent on the development of a broader database of toxic effects on a wider range of ecologically relevant organisms. Extending the range enables better validation of existing soil metal limit recommendations. This will enable the establishment of more effective environmental quality criteria and guidelines, which may be more equitably structured to protect invertebrates important in maintaining ecosystem functioning in habitats threatened by chronic contamination.

G. INVERTEBRATES AS BIOINDICATORS OF METAL CONTAMINATION: A ROLE FOR LARVAL DIPTERA?

Bioindicators are tools to monitor change

A bioindicator is a species used in the detection and identification of disturbances or changes in the quality of the environment (McGeoch 1998). This term has been broadly applied to encompass environmental, ecological and biodiversity indication. To be effective, a bioindicator should provide decision-makers with a tool for planning future management regimes or evaluating current ones.

The utility of bioindicators lies in their predictive capacity, especially in laboratory-based toxicological studies, where the relationship is examined between a substrate contaminant and the levels of effect on an indicator species. The collation of such laboratory-based toxicity studies (which often feature a variety of techniques and a limited range of test species) enables the estimation of risk parameters and establishment of threshold levels, which serve as baselines for tolerance and subsequent projections of potential ecological effects (Will and Suter 1994). For example, toxicity studies assessing trace metal uptake by organisms quantify the net balance between the rate of influx from both dissolved and particulate sources and the rate of efflux from the organism. Influx and efflux rates, as well as the capacity of an organism to detoxify metals, has been shown to vary according to the species examined (Brown 1982).

Invertebrate bioindicators have also been used in field-based assessments, where the outcomes are quantified by community structure, biodiversity and demographic parameters, or correlated

with a specified environmental variable. Examples of invertebrate groups used as bioindicators in the field include crickets, earthworms and grasshoppers (Brieger *et al.* 1992), web and ground spiders (Larsen *et al.* 1994, Marc *et al.* 1999), isopods (Paoletti and Hassall 1999) and myriapod communities (Grelle *et al.* 2000).

Bioindicators in toxicity tests

In contrast to the variety of invertebrates used in the field, laboratory-based toxicology studies have been predominantly conducted on earthworms, due mainly to their ease of manipulation, rapid generation period and size. They have been used extensively to establish metal threshold levels and metal limit guidelines (Will and Suter 1994).

For example, the earthworm *Eisenia fetida* (Annelida: Lumbricidae), exposed to Zn contaminated soils for 56 days, returned an LC_{50} at 745 ppm (Spurgeon *et al.* 1994). (LC_{50} refers to the lowest concentration at which there is a 50% or greater reduction in survivorship). Previously, Neuhauser *et al.* (1984) had reported an LC_{50} of 662 ppm using the same species and metal contaminant. These values (and others from the literature) were used to establish a benchmark of 200 ppm, although confidence in this value is low because of the limited amount of data which could be correlated (Spurgeon *et al.* 1994). Benchmarks are "intended to be thresholds for significant effects on growth and production" and a detailed explanation of their derivation is provided by Will and Suter (1994).

The methodologies employed in toxicity tests are varied

The methodologies employed to invoke toxic responses using soil invertebrates vary, including the type of soil substrate and method of amendment. A variety of substrates, including artificial soil (Neuhauser *et al.* 1984, van Gestel *et al.* 1993, Spurgeon *et al.* 1994), manures (Malecki *et al.* 1982) and agricultural soils (Ma 1982, Streit and Jaggy 1983, Parmelee *et al.* 1997) have been used in laboratory studies. A further refinement is the use of soil substrates taken from contaminated sites and used as exposure substrates for laboratory-grown species (Spurgeon and Hopkin 1996).

The addition of metal salts to a soil or food substrate (known as "spiking") has been widely practiced (Will and Suter 1994, Reinecke and Reinecke 1996). Depending on the method of uptake of the test organism, either the food substance is soaked in the metal salt (Drobne and Štrus 1996, Gräff *et al.* 1997), or the contaminant is added directly to the soil (Ross *et al.* 1981) to achieve the desired concentration.

Warnings have been made against the direct extrapolation of effects on soil biological and biochemical responses where metals are added as a salt, as a considerable proportion of biochemical inhibition can be attributed to soil acidification rather than a direct metal effect (Speir *et al.* 1999). This may have a profound impact on the results of experiments using a test species with a high probability of uptake by direct body contact (such as earthworms) in metal-salt amended toxicity studies. It draws into doubt the reliability of recorded responses using this technique as a generalization for other taxa.

Recent papers have also addressed the issues of "spiking" of biosolids and the contrast between "total" and "biologically active" heavy metals in soils (Yeates *et al.* 2003, Percival 2003). That research reinforces the importance of developing guidelines for the upper limit concentrations for total heavy metals which take into account soil biological and chemical processes.

Chronic toxicity tests and periods of sensitivity

Further difficulties encountered in toxicity studies hinge on the interpretation of short-term (sub-chronic) and long-term (chronic) effects and their extrapolation to the field. The term "chronic" is relative and may represent hugely varying time frames, depending on the species involved (Ecobichon 1992). Chronic studies also need to consider the critical periods of exposure in life-history stages.

For example, an LC_{50} of 16.3 µg/L (copper) and 36.8 µg/L (zinc) recorded for *Tanytarsus dissimilis* (Diptera: Chironomidae) during embryogenesis was substantially lower than other aquatic larvae exposure records. These results were attributed to species specificity and the sensitivity of the developmental period (Anderson *et al.* 1980).

Chronic toxicity in soils receiving biosolids

The chronic accumulation of metals in soils via, for example, successive applications of sewage sludge, may further compound the evaluation of metal toxicity to the soil fauna (McLaren *et al.* 1994). The associations between metals and organic matter in sludge may alter in response to local physical, chemical and microclimatic conditions, thereby influencing metal speciation and its relative bioavailability (van Straalen and Bergema 1995, Ghosh and Banerjee 1997, Posthuma *et al.* 1997).

Whole body metal concentrations are only part of the story

Metal sequestration and detoxification are understood to be energetically costly (Donker 1992, Donker *et al.* 1993, Drobne and Štrus 1996, Spurgeon and Hopkin 1996). Yet this capacity enables an invertebrate to effectively inactivate potentially toxic metals ingested during feeding or absorbed via the epidermis (Hopkin 1990). It provides a degree of plasticity to species encountering metal concentrations above background levels, but does cast doubt on the simple quantification of whole body metal concentrations (WBMC) (sensu Gräff, *et al.*, 1997) as a measure of effect.

The WBMC may indicate uptake parameters and thus, bioavailability, but alone may be a poor indicator of the relative effect of the metal species in the habitat. It is therefore necessary to link WBMC with additional parameters, such as mortality, growth, community structure and abundance to provide a more complete analysis of effects.

The immobilization of surplus metals by invertebrates

It is well-recognized that many invertebrates have the capacity to sequester and detoxify metals accumulated from the environment (Dallinger 1994, Morgan *et al.* 1999). This capacity enables the organism to tolerate relatively high environmental metal loadings. For example, the endogeic earthworm *Aporrectodea calignosa* (Annelida: Lumbricidae) accumulates metals in the posterior alimentary canal where compartmentalization of metals in intracellular vesicles (the chloragosomal matrix) appears to prevent the general dissemination of metals to the whole body and may represent "...a detoxification strategy based on accumulative immobilization..." (Morgan and Morgan 1998). Isopods (particularly *Oniscus asellus* and *P. scaber*), are also well-known for their metal accumulating abilities, with the main site of accumulation being the hepatopancreas (Wieser and Klima 1969, Storch 1984).

Methods of detoxification employed by the Diptera

Early ultrastructural studies of the mid-gut of larval *Drosophila* (Diptera: Drosophilidae) found copper concentrated in granules in cuprophilic cells (Filshie *et al.* 1971). The granules were subsequently examined histochemically and found to be cytolysosomes (Tapp and Hockaday 1977). Lysosomes intervene in the detoxification process in insects exposed to heavy metals; metals may be trapped within lysosomes by a metallothionein (Dallinger 1994). Cytolysosomes are secondary lysosomes, in which segregated cytoplasmic areas with organelles or inclusions are digested. The cup-shaped cuprophilic cells are also involved in acid secretion (Dubreuil *et al.* 1998).

Seeking alternative species for toxicity tests

Although earthworms provide a good baseline, the acknowledged variability of responses within invertebrate groups and between species (Roth 1992, Parmelee *et al.* 1997) calls to question the wholesale use of earthworm-derived toxicity benchmarks. There is merit in the use of a broader range of indicator species, each offering diverse physiological and behavioral characteristics, which can have a substantial bearing on their metal accumulation strategies.

Examples of alternative species used in toxicity studies include woodlice (Drobne and Hopkin 1994, Jones and Hopkin 1998), springtails (Bengtsson *et al.* 1985, Cole *et al.* 2001), millipedes staphylinid beetles (Bohac 1999) and nematodes (Kammenga *et al.* 1994). Unfortunately, the distinct lack of comparability between differing taxa tends to limit the effective assessment of metal toxicity (Gräff *et al.* 1997).

Are the soil-dwelling Diptera ecologically relevant bioindicators?

The association of moisture-dependent species with sewage sludge applications has been reported for some dipteran families, including the Sciaridae and other Nematocera (Hoevemeyer 1999). In that study, it was noted that adult emergence was only a weak indicator of dipteran diversity and suggested the abundance of trophic groups of soil-dwelling dipteran larvae maybe a more sensitive measure.

Soil-dwelling Diptera are known to constitute a significant proportion of the fauna in a variety of ecosystems (see review by Frouz, 1999). In that paper, the Diptera are identified as an important part of the edaphon and may represent the most abundant part of soil macrofauna in some ecosystems; they are also a functionally diverse group. Their potential as bioindicator species has not been widely examined (possibly due to difficulties with larval stage determination), despite their varied trophic status (Smith 1989) and acknowledged role in decomposition and nutrient release (Perel *et al.* 1971).

However, terrestrial dipteran larvae, including *Musca domestica* (Muscidae), *Drosophila melanogaster* (Drosophilidae) have been used for many years in the examination of cellular-level and chemical responses to specific contaminants (Sohal *et al.* 1976, Tapp and Hockaday 1977, Massie *et al.* 1984, Dubreuil *et al.* 1998). Dipterans, as for many other invertebrate groups, have a capacity for metal sequestration (Brown 1982, Simkiss and Taylor 1989).

Although the location of metal-containing granules in invertebrates varies, the organs associated with digestion, storage and excretion processes are most commonly involved (Poulson *et al.* 1952). However, metal accumulation sites and sequestration processes are also known to be taxon-specific (Brown 1982). There also exist biological systems at the cellular level for the uptake and regulation of essential metals, such as copper, although other non-essential metals, such as cadmium, appear able to utilize these routes (Anderson *et al.* 1980, Spurgeon *et al.* 1994). In the majority of invertebrate tissues, the lysosome system appears to be one common link in the pattern of metal accumulation. Although a wide variety of cell structures contain metals, most organelles may be incorporated within a scheme of lysosome origin and function.

The effective identification of specialist sites of accumulation requires specialist equipment and techniques (Borovansky 1997). For example, X-Ray microanalytical mapping of the intracellular distribution of pollutant metals in isopods has enabled the development of models to predict intracellular detoxification pathways (Hopkin 1989). The histological examination of granular inclusions in tissues has been used as an indicator of contamination and metal tolerance in several species.

More straightforward (and less costly) histological techniques for the detection of chronic metal damage at the tissue level may be used to demonstrate treatment effects. Possible effects which can be linked to chronic levels of metal contamination include, for example alterations to the thickness of epithelial tissue and the lumen of malpighian tubules, the number and distribution of vacuoles in fat tissue, and degenerative changes in the basal membrane (Filshie *et al.* 1971, Sohal *et al.* 1976, Doughtie and Ranga Rao 1984, Bischof 1995, Marigomez *et al.* 1998).

H. CONCLUDING REMARKS

In the search for a compromise between economics and ecology, it is necessary "...to identify areas or points of overlap in the dual role of systems..." (Rapport *et al.* 1998b). The human-derived objective of productivity in plantation forest systems is ultimately dependent on soil nutrient availability. Management options employed to enhance nutrient status may interfere with the ecosystem processes that facilitate nutrient cycling. The extent to which biosolids applications might disturb the normal functioning of the invertebrate biota in *P. radiata* plantations in central Canterbury is unexplored. This situation is of both applied and theoretical interest.

It is of applied interest, because a clear understanding of the effects of biosolids applications on biodiversity may be useful in evaluating whether the Christchurch "Biosolids to Forests"

programme should be continued, amended or extended. A better understanding could also be useful where similar projects are proposed elsewhere. It is of theoretical interest, because it provides a setting in which to examine a number of questions related to biodiversity, ecosystem function, habitat heterogeneity, and species-level sensitivities.

CHAPTER THREE

THE DIVERSITY OF SOIL AND LITTER-DWELLING ARTHROPODS IN A *PINUS RADIATA* PLANTED FOREST AND THE EFFECTS OF BIOSOLIDS APPLICATION

A. INTRODUCTION

One of the keys to the sustainable management of planted forests has been said to lie fundamentally at the level of the soil (Nambiar 1996, Herrick 2000, Setälä *et al.* 2000). Intimately associated with the soil and the overlying litter substrate, is a diverse biotic component, participating in and providing, a raft of ecological processes and services (Anderson and Ineson 1984, Seastedt 1984, Usher 1985, Jones and Lawton 1995, Bengtsson 1998, Brussard 1998). The micro- and macroarthropods, which constitute a substantial fraction of the biomass in this habitat, are major regulators of nutrient cycling (Giller 1996). Management activities which result in the modification of soil fauna communities can bring about losses of diversity, which in turn may result in the loss of specific structural patterns or regulatory mechanisms (Lavelle 1997).

Tree growth is dependent on the nutrient status of the soil (Hart and Firestone 1991, Miller 1995, Heneghan and Bolger 1998). As soil arthropods are responsible (at least in part) for the local mobilization of nutrients, it has been argued that tree growth (especially in the first 3-5 years) could be compromised by small-scale changes in the diversity and functional architecture of soil arthropod assemblages (Haskell *et al.* 1992, Bardgett and Cook 1998, Rapport *et al.* 1998b). If this is so, local site productivity may ultimately be affected.

An integral part of holistic ecosystem management for at-risk sites, is inventory collation and the monitoring of biodiversity for possible shifts in community structure (Kremen *et al.* 1992). The methodologies used for biodiversity assessment are many and varied. Although diversity indices, such as Shannon-Wiener (H') were once common as a stand-alone measure (Gotelli and Colwell 2001), ecologists have increasingly used trophic status, or allocation to functional group or indicator species analysis in biodiversity assessment (Schulze and Mooney 1993, Lawton and Brown 1994, Jones and Lawton 1995, Dufrene and Legendere 1997). These methods of assessment consider not only the diversity of the components of a system, but also enables focus on what species do in ecosystems. However, irrespective of the means, effective

management for biodiversity is underpinned by the need to both document the biotic status of a site and provide recommendations on how to maintain or enhance that diversity.

Do biosolids applications to planted forests constitute a risk to the diversity of invertebrates and their contribution to essential ecological services? How resilient and resistant is the community? Can it be shown that the application of biosolids conforms to sustainable practice which advocates the avoidance of land-use activities that may compromise local diversity and the viability of species and their habitats (Elliott and Lynch 1994, McCracken and Bignal 1998)?

The objective of this chapter was to collate a multi-season taxonomic inventory to characterize the soil and litter dwelling invertebrates associated with two *Pinus radiata* planted forests in mid-Canterbury, New Zealand. Both sites had previously been selected for the long-term monitoring of environmental effects for the CCC's "Biosolids to Forests" programme. A subsequent, short survey of these sites, approximately six months after the application of biosolids, sought evidence for change in the invertebrate species assemblage, at individual, taxon and functional levels of resolution.

The invertebrate fauna associated with planted forests in this locality had not previously been documented. Earlier studies in other planted forests across New Zealand had described a distinctive, if limited assemblage (Styles 1967, McColl 1974b, Johns *et al.* 1980, Hutcheson *et al.* 1999). The application of biosolids present novel physical and chemical conditions for soil and litter invertebrates; the extent to which an application programme may impact on local biodiversity is largely unexplored.

The first hypothesis for the post-treatment survey was that biosolids applications would alter arthropod abundance. This is because the biosolids were expected to provide additional resources (primarily nutrients) to the system which could variably favour or compromise arthropods, according to their capacity to utilize the resource. The second hypothesis was that biosolids applications would alter the proportional abundance of arthropods in functional groups. The third hypothesis was that biosolids applications would alter arthropod diversity. This is because the biosolids were expected to provide additional niches for potential colonizers. Thus, a greater diversity of species were expected to be present in treated plots than untreated plots.

The outcomes were expected to (i) supplement the limited entomological data available about arthropod diversity in these exotic forests; (ii) provide evidence of a biosolids-mediated change in community trophic structure and diversity; and (iii) identify potential indicator species and assemblages suitable for future investigations and to facilitate planning for the sustainable management of the Christchurch City Councils' "Biosolids to Forests" programme.

B. METHODS

1. Study sites

The long-term monitoring sites, Hunter's Road (HR) and Doyle's Block (DB), are located west of Dunsandel, approximately 50 km south-west of Christchurch in the mid Canterbury region of the South Island of New Zealand (Figure 3.1).

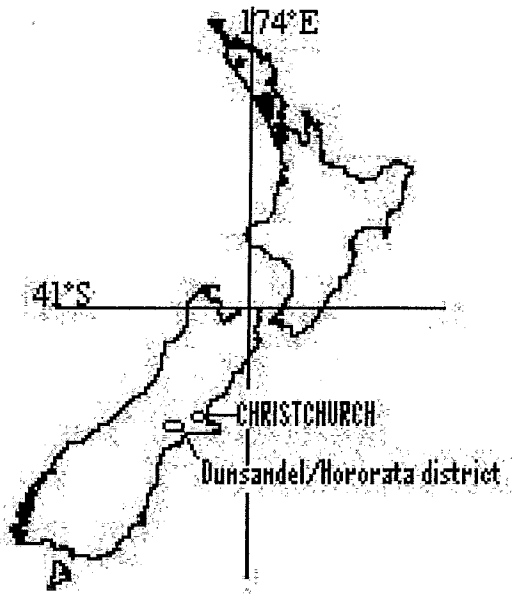

Figure 3.1 Study site location in the Hororata district, approximately 50 km south west of Christchurch, New Zealand.

The Hororata district has a flat topography and the dominant soil types are Balmoral stony and very stony silt loams and Lismore shallow and stony silt loams; soil pH varies between 4.7 and 5.2 units (D.S.I.R. 1968). Climatic data for this district was obtained from the Hororata weather

station. (Appendix Table A1). This area receives < 600 mm rainfall per annum. The sites are described as follows:

(i) Hunter's Road study site. This is a 115.06 ha block located on Hunters Road, on the south side of the Hororata-Dunsandel Road. This second rotation (R2), 4 year-old *P. radiata* forest was planted in 1994 at a density of 1250 stems/ha. The trees were grass-released with herbicide in 1994 and 1995 and flail-released in April 1997 and May 1999 for control of woody weeds. At the time of sampling no needle litter had accumulated on the forest floor and the mineral soil was exposed underneath most of the tree lines. The patchy vegetation, which included unidentified pasture grasses and common woody weeds (e.g. gorse, broom, wattle, lavender) was interspersed with bare ground. The gorse and broom grew in thick, low clumps (< 30 cm high) however the wattle grew to 2 m high prior to flailing.

(ii) Doyles Block study site. This is a 165.60 ha block located on Mitchell's Road on the south side of the Hororata-Dunsandel Road. This 20-year old R2 *P. radiata* forest was planted in 1978 at a density of 1250 stems/ha. The trees were band sprayed for grass release in 1978 and low pruned and waste thinned in 1985. Throughout the forest, pruning waste and needle fall form a continuous litter layer, up to 5 cm deep. The forest supports a limited understorey plant community, composed of scattered gorse and broom, primarily around the immediate perimeter of the forest, and very occasionally within gaps formed by wind throw.

2. Plot layout

At both monitoring sites, 9 permanent, independent sampling plots were pegged, each consisting of a 20 m x 20 m sampling area, bordered by a 10 m buffer. Buffer perimeters at DB were separated by windrows. The plots at HR were spaced longitudinally and flanked by windrows; the minimum distance between plots was 40 m.

3. Identification of invertebrates

The New Zealand invertebrate fauna is diverse and poorly known. Taxonomic limitations and the lack of available expertise for some taxa necessitated and justified the use of Recognizable Taxonomic Units (RTU) (Oliver and Beattie 1993). For both the characterization inventory and the post-treatment survey, specimens were sorted to species or RTU using reference specimens from the Lincoln University Entomology Museum, in addition to keys for the Arachnida (Forster 1967, Forster and Wilton 1968, Forster 1970, Forster and Wilton 1973, Forster and

Blest 1979, Forster *et al.* 1988), Opiliones (Forster 1954), Coleoptera (Hudson 1975, Stibick 1979, Johnson 1982, Klimaszewski and Watt 1997)and Diplopoda (Johns 1962, Johns 1964). The Collembola were only identified to Order. In some samples, it was not possible to obtain an accurate count and the values given are an estimate. The Pseudoscorpionidae were not taken beyond ordinal level, whilst the mites within the Acari were identified only to family level. The Staphylinidae (Coleoptera) were not taken beyond sub-family level. It is also acknowledged that the nematode and enchytraeid fauna expected to be present in the forest soils were unlikely to have been effectively accounted for using the trapping methodologies employed in this study. As they have been examined in depth in other *P. radiata* ecosystems in New Zealand (Yeates 1989, Yeates *et al.* 2000). they are not considered further in this thesis, although it is acknowledged they are an important group in soil ecology.

4. Survey 1: Characterization Inventory

4.1 Temperature

A portable temperature probe was used to measure temperature at 2 m intervals along a 10 m mid-row transect at both HR and DB. Each of the 5 measurements was made at three depths (surface, 5 cm and 10 cm) on the same data. Data are given as the mean values (± SEM) for each site and intended only as a snapshot comparison between sites on one day during each sampling period. Measurements for both sites were made between 12 noon and 2 pm.

4.2 Sampling methodologies

Three methods of sampling (one of each type) were used in each of the nine plots at each site.

(i) Pitfall traps. These are a simple and effective means of accounting for surface arthropod abundance and activity. Where used continuously, they are a reliable relative measure of the population size of both carabids and spiders (Southwood 1966, Meijer 1974, Uetz and Unzicker 1976, Baars 1979) although they may tend to over-represent the more active species (Cole *et al.* 2001). Other limitations associated with pitfall trapping have been well discussed (Spence and Niemala 1994). It is noted that pitfall trapping entails destructive sampling. To minimize this effect, only one pitfall was placed in each plot. Pitfall traps consisted of a funnel (6 cm diam.) made from a PET bottle cut in half. A 250 ml jar containing a preservative (70% ethylene glycol) was placed within each trap. Each pitfall was placed approx. 10 m from the centre of the plot. The trap was buried flush with the ground and protected by a 12 cm x 12 cm plastic lid fixed 10 cm above the trap aperture. Traps were checked weekly and extra preservative (100% ethylene glycol) added, especially where trap catch was high, to

maintain a viable preserving solution. At the completion of each 4-week sampling period, the traps were cleared and closed. Data are reported as the total number of individuals trapped from a total of 9 plots for each sampling period at the two sites.

(ii) Water pan traps. These are a useful trapping methodology because many invertebrates are attracted to white objects, and readily gather around moist sites; water traps also account for mobile species which inadvertently leap into the water (New 1998). Traps consisted of a shallow, white plastic dish (15 cm x 10 cm x 4 cm). White was the default colour choice although it is acknowledged that yellow also works well as an attractant. The dish was sunk flush into the ground approx. 10 m diagonally opposite each pitfall trap. Each trap was filled with water, plus a few drops of unscented detergent (a surfactant to break the surface tension) and 5g common salt (a preservative). All traps were emptied weekly to limit decomposition, contamination of the sample by needle fall and dirt and to replace evaporative loss. Invertebrates were transferred from the pan and stored in 70% alcohol prior to sorting and identification. Data are reported as the total number of individuals captured from the 9 plots for each sampling period at the two sites.

(iii) Litter/soil sampling: Berlese-Tullgren extraction is a well-established method of removing micro- and macro-arthropods from a standard volume of litter material (New 1998). The method relies on the negative taxis of arthropods from heat and light. The equipment for this study utilized two simple extractor boxes enabling 6 samples to be processed simultaneously. Litter was placed underneath a suspended light bulb (40 watts) on a mesh diaphragm (5 mm x 5 mm) supported by a funnel which opened into a collecting vial of 70% ethanol. Extraction funnels may poorly account for some juvenile and larval stages because of limited mobility, susceptibility to desiccation or inappropriate mesh size (New 1998). Similarly, the volatiles released from alcohol preservatives may "repel" animals. In an attempt to increase the humidity, several glass jars containing water were placed in each extractor box. Litter samples were taken weekly from transects in each plot during each sampling period at each site (HR and DB). A weekly litter sample consisted of 5 placements of a 10cm x 10cm metal grid at 1m intervals from which all litter plus mineral soil to a depth of 5cm was removed.. Where woody vegetation at HR coincided with a quadrat placement, the sample was taken from the closest adjacent non-vegetated area. The total volume of material removed from each plot during each weekly sample varied according to the amount of litter on the surface of the forest floor. For this reason, the 5 samples from each

replicate plot were immediately bulked (into a very large clear plastic bag) to give a sample representative of each plot. The bags contained ample oxygen and were sealed and then stored in a darkened cool room at 4°C. Bags awaiting extraction were regularly opened to refresh gases and gently tumbled prior to taking sub-samples for processing. Six sub-samples were taken from each bag on a random basis and processed for 4 days in the Berlese-Tullgren apparatus. The volume of each sub-sample taken was sufficient to cover each mesh grid (18cm diameter) to a depth of 2cm. The processing period for the entire litter sample lasted 36 days. Invertebrates were stored in alcohol prior to sorting and identification. Data are reported as the total number of individuals captured from the 9 plots for each sampling period at the two sites. Data are reported as the total number of individuals trapped from the 9 plots for each sampling period at the two sites.

4.3 Sampling interval

Both HR and DB were sampled at two-monthly intervals between mid-December 1998 and mid-January 2000. Each sampling period was for four consecutive weeks. This time frame took into account both the diurnal and seasonal variation in arthropod activity patterns and provided the maximum opportunity to account for species phenologies. A conservative approach was taken in relation to the sampling intensity within plots, given that all methods used were destructive. It is possible that the data under-represents the true abundance and community composition of invertebrates, because extensive earthworks using heavy machinery to install lysimeters in each plot during the survey period disturbed the forest floor surface within every plot. These disturbed areas were not directly sampled.

5. Survey 2: Post-treatment survey

During September, 2000, a purpose-built tractor trailer unit was used to apply dewatered biosolids to the permanent plots within the two monitoring sites at rates equivalent to Nil, 400 and 800 kg N/ha. The Nil biosolids plots at HR were systematically passed over with the tractor trailer unit however this was not logistically possible at HR. The following February, 2001, both HR and DB were surveyed using only pitfall trapping for a period of four consecutive weeks. This period represented mid summer. Short sampling periods in the warmest months in New Zealand have been shown to be effective in accounting for invertebrate activity in plantation forests (J. Hutcheson, pers.com.). One pitfall trap was placed in each of the 9 plots at each site, and each pitfall was at least 5m away from where a pitfall had been placed for the previous Characterization Survey 1. Arthropods were removed from traps weekly, when the

preservative within each trap was recharged. The catch was sorted for each week and stored as described previously. It is acknowledged that the sampling methodology was destructive and continuous. For this reason, the total catch from each trap in each plot was treated as 1 sampling unit. Each of the 3 replicate plots for each of the 3 biosolids treatment rates at each site was represented by 1 pitfall sampling unit. Each pitfall trap constituted a total of 672 trap hours. Data are presented as the mean abundance of taxa from each of the three treatments.

6. Presentation and analysis of the two surveys

The characterization inventory is presented as a taxonomic list identifying the total abundance of invertebrates in relation to the trapping methodology and seasonal occurrence. This enabled comparisons to be drawn between trapping methodologies, site and seasonal as well as the representation of indigenous, rare and introduced taxa at each site.

The post-treatment survey data was examined in the following order:

(i). Count data were tabulated to show the mean abundance of all families, taxa and individuals in each of the three biosolids treatments (Nil, 400 and 800 kg N/ha).

(ii) The raw abundance count data for families, taxa and individuals within each of the biosolids treatments was transformed [$\sqrt{(x+0.5)}$] prior to conducting a stepwise ANOVA on each of these three taxonomic categories at each site.

(iii) An additional stepwise ANOVA of the transformed data was undertaken in which the data was analysed both with and without the individual counts for the Collembola. This was undertaken to identify a possible cause for an observed increase in the abundance of arthropod individuals in the 400 kg N/ha treatments.

(iv) All arthropods, with the exception of the Collembola, were then allocated to a functional category, based on the observation of mouthparts and published information on the biologies of the species. Although categorization into functional groups facilitates broad generalizations about assemblages, it is noted that the approach is limited in situations where the biology of the individual is poorly understood, or if proper account is not taken of the variety of life stages exhibited and their potentially different use of food resources. Six arbitrarily selected functional groups were used, including mycetophagous, phytophagous, predatory, scavenger, omnivorous, and non-feeding. The latter functional group was used because it accounted for the adult craneflies which were present and are known to seek only moisture. The Collembola presented a difficulty with regards to allocation to functional group. Collembola are gregarious in their feeding preferences and even at species level may exhibit variable feeding preferences (Greenslade 1996). As the Collembola were only identified to order they were not included in

the functional level analysis presented for the post-treatment survey. It is acknowledged their exclusion may have altered the outcomes. The percentage of (i) individuals and (ii) taxa in functional groups in each plot was calculated. The two datasets were then arcsine transformed [arcsine $\sqrt{(x/100)}$] to normalize and examined separately by stepwise ANOVA (PRISM, GraphPad Software, 5755 Oberlin DV, #110, San Diego, CA 92121, USA). The *F* tests were considered significant at $\alpha = 0.05$. Tukey's test was used to identify significant differences between functional groups.

(vi) The arcsine transformed data for functional groups from untreated plots only at HR and at DB was examined by ANOVA in relation to (i) individuals in functional groups and (ii) taxa in functional groups. This analysis was undertaken to characterize the trophic structure of the two sites. Tukey's test was used to identify significant differences between functional groups.

(vi) The Shannon-Wiener diversity index (H') was used to quantify the diversity of the invertebrate assemblage within biosolids treatment rates for the post-treatment analysis. The index is based on information theory and is expressed as

$$H' = -\sum_{i=1}^{S} (p_i)(\log_2 p_i)$$

where H = Index of species diversity, S = number of species and p_i = proportion of total sample belonging to the *i*th species (Krebs 1989). This index combines information on species richness and abundance values in a single number. Diversity values were examined using ANOVA to determine significant differences in the mean H' between biosolids treatment rates at HR and DB.

(vi) The degree of similarity in the species composition among treatments at each site was estimated using Sorenson's Similarity Index (Sim). This provides an index describing beta diversity, which enables comparison of communities under different management systems. Beta diversity is high when Sim. is low, (i.e similarity is lowest when Sim. is low); the higher the Sim., the lower the species' turnover between habitats. Computed values range from 0-1, with a higher value suggesting a higher level of similarity. The equation is expressed as

$$Sim = (2\sum nc)/(\sum n1 + \sum n2)$$

where nc = the number of species in common, n1= the total number of species at site 1 and n2 = the total number of species at site 2.

(vii) The data from each site was examined using Detrended Correspondence Analysis (DCA). Objects are plotted along axes according to their resemblance, enabling data to be reduced and expressed in many dimensions. Correlations in the structure are used to position the objects in space, such that close objects are similar in their ecological affiliations, whilst objects more

distant in the ordination space are loosely associated. In each analysis, the DCA ordination was based on the default setting of 26 segments. The default setting has been criticized (Dufrene and Legendere 1997) because results may vary according to the number of segments used to remove the arch effect associated with Correspondence Analysis (Hill and Gauch 1980). Therefore, several runs were made of the data using a different number of segments to establish stable axes and results which were interpretable (Dufrene and Legendere 1997). The axes were rescaled and the length of the ordination axis was defined to be the range of the site scores, expressed in multiples of the standard deviation (SD) (Hill and Gauch 1980). Sites that differ 4 SD in scores can be expected to have no species in common (Jongman *et al.* 1995). Detrending the axes destroys the correspondence between the eigenvalue and the structure along that axis; thus, eigenvalues cannot be interpreted as the proportions of the variance explained for individual axes.

(viii) The data was then examined using the technique of Dufrene and Legendre (1997). For each site and biosolids treatment rate, Two-Way Indicator Species Analysis (TWINSPAN) was used to generate an hierarchical clustering based on the similarities of taxa occurrence. The initial split generated by TWINSPAN established a dichotomy, in which taxa were associated with either treated or untreated plots. Taxa were labeled according to their association (Group 1 or Group 2). The resulting databases were then examined using the Indicator Species Analysis programme (PCORD). This method ranks taxa and provides probabilities of association; the statistical strength of the taxa as being indicative of a specific treatment within a site is shown. Dufrene and Legendre (1997) define indicator species as " the most characteristic species of each group, found mostly in a single group (within each site typology) and present in the majority of the sites belonging to that group." Indicator species, therefore, are taxa which typify a condition and can be subsequently used to assess changes to that condition. Whilst it is noted that the effectiveness of an indicator species (or suite of species) should be validated, environmental assessment impacts using rapid sampling of key arthropod taxa known to be typical of a location may be more cost effective than detailed collections requiring taxonomic expertise.

C. RESULTS

1. Survey 1: Characterization inventory

1.1 Site microclimate

Season, site and depth-related differences in the mean temperature of the forest floor were found at both HR and DB (Figure 3.2). The mean temperature at the surface of the forest floor

was consistently higher at HR than DB for all sampling occasions. The highest midday temperatures were recorded during summer (Dec/Jan) and the lowest midday temperatures during winter (Jun/Jul). During summer, the midday surface temperature at Hunter's Road was at least 12°C more than at DB. Irrespective of depth, the variation in the mean temperature of soils at midday was less at DB than at HR. At each site, the mean temperature was found to decrease with soil depth.

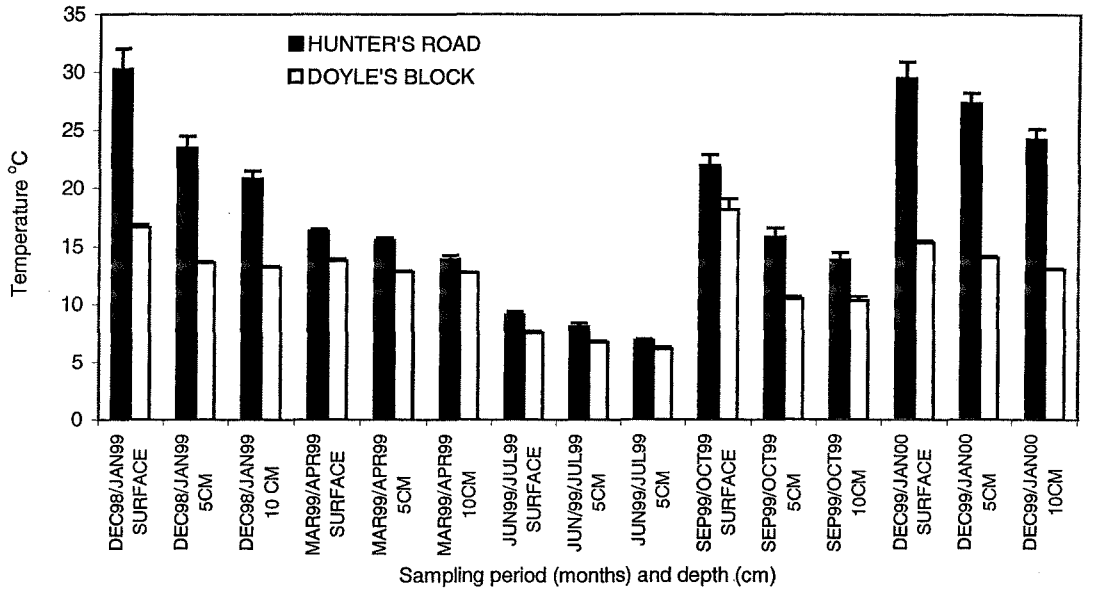

Figure 3.2. Mean (+SD) same-day soil temperature at the surface, 5cm and 10 cm depths at the HR and DB stands for the sampling periods Dec/Jan 1998-1999, Mar/Apr 1999, Jun/Jul 1999, Sept/Oct 1999, Dec -Jan 1999/2000. Values represent the mean of 9 readings for each depth.

1.2 Arthropods

The arthropod orders trapped by a combination of pitfall, pantrap and litter extraction techniques at both HR and DB included representatives of the Acari, Aranaea, Phalangida, Pseudoscorpionida, Hymenoptera, Orthoptera, Coleoptera, Collembola, Dermaptera, Diptera and Myriapoda. The three trapping methods employed differed in the proportion of individuals represented in the catch (Table 3.1). The relative abundance of individuals trapped by either method (pitfall, pantrap, litter extraction) at the two sites was highly variable, indicating a patchy distribution of taxa across plots.

At HR, a total of 5,207 invertebrates were trapped, whilst at DB, 9,893 individuals were accounted for. At HR these individuals constituted 59 families represented by 86 recognizable taxonomic units. At DB, 58 families were represented by 87 recognizable taxonomic units The

four most abundant arthropod groups at both HR and DB included the Collembola, Araneae, Coleoptera and Diptera.

Pitfall traps consistently accounted for a higher proportion of individuals than either pantraps or litter sampling. Because there was almost no pine needle litter at HR, litter sampling accounted for a very low proportion of individuals and number of families at this site, compared with DB. In most cases, the number of families accounted for was lowest in the winter period (Jun/Jul) and highest in the summer period (Dec/Jan).

Table 3.1 The relative abundance (%) of individuals per trap type and the number of families (bold, in brackets) at Hunter's Road and Doyle's Block during each sampling period of the characterization survey.

	HUNTER'S ROAD			DOYLE'S BLOCK		
SAMPLING	Relative abundance of individuals per trap type and **(number of families)**					
PERIOD	**PITFALL**	**PANTRAP**	**LITTER**	**PITFALL**	**PANTRAP**	**LITTER**
DEC98/JAN 99	42 **(25)**	57 **(33)**	1 **(11)**	32.9 **(29)**	50.5 **(31)**	16.6 **(19)**
MAR99/APR99	78.8 **(20)**	19.9 **(15)**	1.3 **(1)**	30.7 **(21)**	33.3 **(23)**	36 **(23)**
JUN99/JUL99	69.6 **(12)**	19.3 **(8)**	11.1 **(3)**	60.9 **(29)**	15.9 **(11)**	23.2 **(10)**
SEP99/OCT99	75.5 **(20)**	22.2 **(3)**	2.3 **(4)**	41.9 **(14)**	12.4 **(19)**	45.7 **(12)**
DEC99/JAN 00	68.8 **(27)**	28.9 **(35)**	2.3 **(6)**	47.7 **(31)**	32.3 **(33)**	20 **(18)**

At HR, the pantraps were especially effective at catching cursorial hunting species, including *Phalangium opilio* and *Lycosa hilaris,* Collembola, the ground-active cricket *Bobilla* sp., the weta *Pleioplectron simplex,* as well as a number of Diptera, represented by the families Anisopodidiae, Anthomyiidae, Asilidae, Calliphoridae, Cecidomyiidae, Dolichopodidae, Drosophilidae, Ephydridae, Mycetophilidae, Phoridae, Stratiomyiidae and Therividae .

At DB, water-filled pantraps effectively accounted for the phalangid *Nuncia* sp., *Lycosa hilaris*, Collembola, the dermapteran *Forficula auriculata,* the isopod *Porcellio scaber* and the cricket *Bobilla* sp., in addition to the dipteran families Anisopodidae, Asilidae, Calliphoridae, Cecidomyiidae, Chloropidae, Dolichopodidae, Drosophilidae, Ephydridae, Mycetophhilidae, Stratiomyidae and Tipulidae.

The proportional representation of indigenous and introduced taxa (where provenance was known with confidence) differed between sites with the exception of the Orthoptera (Table 3.2). Within each of the orders, at least 50% of species were shared. Some forest pest species were present, albeit at a low abundance and were unlikely to represent a forest health threat. Pest species included the coleopterans *Arhopalus tristis, Hylastes ater* and *Hylastes ligniperda.*

A full taxonomic list of individuals trapped from HR and DB is presented in Table 3.3 and Table 3.4 respectively.

Table 3.2. A comparison of taxonomic representation at Hunter's Road (HR) and Doyle's Block (DB) from the characterization survey. * where known with confidence. p = present, a = absent.

ORDER	FAMILIES		SPECIES		Taxa in common	Indigenous species * (%)	
	HR	DB	HR	DB		HR	DB
Acari	2	7			1 Family		
Phalangida	2	2	2	3	2 species		
Pseudoscorpionidea	a	p	a	p	Nil		
Araneae	13	11	24	18	13 species	57	42
Coleoptera	12	14	25	32	18 species	39	38
Collembola	p	p	?	?	?		
Dermaptera	1	1	1	1	1 species		
Myriapoda	4	3	4	3	3 species	50	66
Diptera	13	14	13	16	9 species	87	80
Hymenoptera	3	2	3	2	1 species		
Isopoda	2	2	1	1	1 species		
Orthoptera	5	5	3	3	3 species	100	100

Table 3.3 Inventory of individual abundance within invertebrate taxonomic groups at Hunter's Road. Samples account for all individuals trapped by either pitfall, pantrap or litter extraction. Samples were taken from traps kept open continuously for 4 week periods within the sampling intervals DEC98/JAN99, MAR99/APR99, JUN99/JUL99, SEP99/0CT99, DEC99/JAN00. One trap of each type was placed in each of nine independent 40x40 m plots at each site. *indigenous # introduced (where provenance is known with certainty).

	DEC/JAN 1998-1999			MAR/APR 1999			JUN/JUL 1999			SEPT/OCT 1999			DEC/JAN 1999-2000		
HUNTERS ROAD															
ORDER															
Sub Order															
FAMILY	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter
Species															
ACARI															
Cryptostigmata															
GAMISIIDAE			1												
Mesostigmata															
UROPODIDAE			2						1			1			
PHALANGIDA															
Laniatores															
TRIAENONYCHIDAE															
**Nuncia* sp.										2	61				
Palpatores															
PHALANGIDAE															
#Phalangium opilio Linnaeus	63	367		55	27		12	2		18			126	66	
ARANEAE															
CORINNIDAE															
#Supunna picta Koch L.				2	2		1						2	1	
CLUBIONIDAE															
**Clubiona cambridgei* Koch L.				1											
**Clubiona huttoni* Forster 1979														1	
CTENIDAE															
**Horioctenoides* sp.				1									1		
DIPLURIDAE															
**Aparua kaituna* Forster 1968										2					
DYSDERIDAE															

HUNTER'S ROAD	DEC/JAN 1998-1999			MAR/APR 1999			JUN/JUL 1999			SEPT/OCT 1999			DEC/JAN 1999-2000		
ORDER Sub Order FAMILY *Species*	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter
#*Dysdera crocata*. Koch L.										11			32	5	
GNAPHOSIIDAE															
#*Hemicloea rogenhoferi* Koch L.		1												1	
**Taieria erebus* Koch L.	1		1												
**Anzacia gemmea* Dalmas	5	1											11	5	
**Nauhea tapa* Forster														2	
LINYPHIIDAE															
Mynogleninae RTU 1		2											111	16	
#*Diplocephalus cristatus* Blackwall	1									4			109	6	
#*Lepthyphantes tenuis* Blackwall				7			8			3			1	3	1
#*Microtenonyx subitaneus*										21					
#*Ostearius melanopygius* Cambridge														1	
LYCOSIDAE															
**Lycosa* n.sp.	4				3					4			6	8	
**Lycosa hilaris*	197	14		15	36		18			57			154	47	1
MICROPHOLCOMMATIDAE															
Micropholocommatidae RTU1							5								
OXYOPIIDAE															
**Oxyopes gracilipes* White	2													1	
PSECHRIDAE															
**Poaka graminicola* Forster & Wilton							1								
SALTICIDAE															
Holoplatys sp.	1												1	1	
THERIDIIDAE															
#*Achaeranea verruculata* Urq.														2	
#*Steatoda capensis* Hann													2	2	
**Steatoda lepida* Cambridge										1			1	4	

HUNTER'S ROAD	DEC/JAN 1998-1999			MAR/APR 1999			JUN/JUL 1999			SEPT/OCT 1999			DEC/JAN 1999-2000		
ORDER Sub Order FAMILY *Species*	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter
COLEOPTERA															
ANTHICIDAE															
#*Trichananca fulgida* Blackburn		1	1										1		
SCARABAEIDAE															
#*Aphodius tasmaniae* Hope 1846	2	3		1											
BRENTIDAE															
Apioninae RTU1									3	3					
#*Exapion ulicis* Forster									1	1	3				
CARABIDAE															
#*Hypharpax australis* DeJean 1828	2									1					
#*Laemostenus complanatus* Dejean	4			29	4			7		11			13	10	
**Megadromus antarcticus* Chaudoir	1	2		5											
**Metaglymma moniliferum* Bates	1														
**Mecyclothorax rotundicollis* White 1984.				1									8	1	
CERAMBYCIDAE															
**Adriopea* sp.					13		1						2		
COCCINELLIDAE															
#*Coccinella undecimpunctata* L.	2												1		
ZOPHERIDAE (COLYDIIDAE)															
**Pristoderus antarcticus* White				1											
**Pycnomerus sophorae*													2	1	
CURCULIONIDAE															
Curculionidae RTU1										3					
Curculionidae RTU2													1		
Pentarthrum sp.														1	
#*Otiorhyncus ovatus* Linnaeus	12	2		44	1								14	7	
#*Steriphus diversipes* Pascoe			1	1						1			9		1
#*Hylastes ater* Paykull				29											

HUNTER'S ROAD	DEC/JAN 1998-1999			MAR/APR 1999			JUN/JUL 1999			SEPT/OCT 1999			DEC/JAN 1999-2000		
ORDER Sub Order FAMILY *Species*	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter
ELATERIDAE															
#*Agrypnus variabilis* Candeze	16	21								18			2		
#*Conoderus exsul* Sharp	21	1		1									7		
HISTERIDAE															
Histeridae RTU1													3		
OEDEMERIDAE															
**Selenopalpus aciphyllae*	5	4													
STAPHYLINIDAE															
Oxytelinae								1					8	2	
Staphylininae					4								3		
COLLEMBOLA	64	80	5	50	22	16	32	12	10	22	20	5	50	15	24
DERMAPTERA															
FORFICULIDAE															
#*Forficula auricularia*	40	27	3	38			8			36			67	37	
MYRIAPODA															
Chilopoda															
HENICOPIDAE															
**Lamyctes emarginatus* Newport 1844	10			1									38	1	
DIPLOPODA															
JULIDAE															
#*Cylindroiulus britannicus* Verhoeff 1891							6			56			8		
#*Ophyiulus pilosus* Newport 1842										1					
DALODESMIDAE															
**Icosidesmus variegatus* Carl 1902										2					
SCHEDOTRIGONIDAE															
**Schedotrigona* sp.										1					
DIPTERA															
ANISOPODIDAE															

HUNTER'S ROAD	DEC/JAN 1998-1999			MAR/APR 1999			JUN/JUL 1999			SEPT/OCT 1999			DEC/JAN 1999-2000		
ORDER Sub Order FAMILY *Species*	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter
**Sylvicola* sp.		10			4									15	
ASILIDAE															
**Sarapogon* sp.	5	12			6								1	13	
CALLIPHORIDAE															
#*Lucilia sericata*	12	9		4	1							2		2	5
CECIDOMYIIDAE															
CecidomyiidaeRTU1		4	2											3	7
DOLICHOPODIDAE															
Micropygus sp.		6												2	
DROSOPHILIDAE															
Drosophila sp.		3											1	1	
EPHYDRIDAE															
**Psilopa huttoni* Hendel		1													
MYCETOPHILIDAE															
**Mycetophila* sp.		28												7	
**Zygomyia* sp.		3												4	
PHORIDAE															
Phoridae RTU1		2												1	
**Melangyna novaezealandiae*	1	15	2		1									4	
THERIVIDAE															
**Anabarrhyncus* sp.		1													
TIPULIDAE															
larvae		1										1			
HYMENOPTERA															
ICHNEUMONIDAE															
Ichneumonidae RTU1					1										
POMPILIDAE															
**Priocnemis* sp.		22	1	1										6	

HUNTER'S ROAD	DEC/JAN 1998-1999			MAR/APR 1999			JUN/JUL 1999			SEPT/OCT 1999			DEC/JAN 1999-2000		
ORDER Sub Order FAMILY *Species*	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter
APIDAE															
#Apis mellifera	25	83		7	15								26	130	
ISOPODA															
ARMADILLIIDAE															
#Armadillium vulgare							1	1							
#Porcellio scaber Latreille 1804	32	7	1	6	3		1	1					2	10	
ORTHOPTERA															
ACRIDIDAE															
**Phaulacridium marginale* Walker 1870														2	
ANOSTOSTOMATIDAE															
**Hemiandrus* sp.	2	4						1						8	
GRYLLIDAE															
**Bobilla* sp.	116	142		673	103					10			392	51	
RHAPHIDOPHORIDAE															
**Pleioplectron simplex* Hutton 1897	69	58						1		1					
TETTIGONIIDAE															
**Conocephalus bilineatus* Erichson 1842	1	2		1										1	

Table 3.4 Inventory of individual abundance within invertebrate taxonomic groups at Doyle's Block. Samples account for all individuals trapped by either pitfall, pantrap or litter extraction. Samples were taken from traps kept open continuously for 4 week periods within the sampling intervals DEC98/JAN99, MAR99/APR99, JUN99/JUL99, SEP99/0CT99, DEC99/JAN00. One trap of each type was placed in each of nine independent 40x40 m plots at each site. *indigenous # introduced (where provenance is known with certainty)

	DEC/JAN 1998-1999			MAR/APR 1999			JUN/JUL 1999			SEPT/OCT 1999			DEC/JAN 1999-2000		
DOYLE'S BLOCK															
ORDER															
Sub Order															
FAMILY	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter
Species															
ACARI															
Cyptostigmata															
GAMISIIDAE	1		8	2		4	6		2	12		4	2		10
CHAMOBATIDAE	1		4	2		7	2		13		3	14			3
NEOTRICHOZETIDAE															
Neotrichozetes RTU1	1					217	25						14		44
Mesostigmata															
PARASITIDAE			4			7	1		8			6			11
UROPODIDAE			4			2	3		1			8			10
Prostigmata															
Prostigmata RTU1			3			1			12						7
CUNAXIDAE			1			2			1				2		1
PHALANGIDA															
Laniatores															
TRIAENONYCHIDAE															
Triaenononychidae RTU1												1		2	
**Nuncia* sp.	83	54			6	2	11	37		13	6		232	123	
Palpatores															
PHALANGIDAE															
#*Phalangium opilio*					60										
PSEUDOSCORPIONIDA			1			5			1						4
ARANEAE															
ANAPIDAE															

DOYLE'S BLOCK	DEC/JAN 1998-1999			MAR/APR 1999			JUN/JUL 1999			SEPT/OCT 1999			DEC/JAN 1999-2000		
ORDER Sub Order FAMILY *Species*	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter
**Chasmocephalon armatum* Forster	10														
CLUBIONIDAE															
**Clubiona cambridgei* Koch L.	2	1		3											
**Clubiona huttoni*	12	1		3											
**Clubiona peculiaris* Koch L.													2		
CORINNIDAE															
#*Supunna picta*					2										
DIPLURIDAE															
**Aparua kaituna*		1		6	4	2		4		26			1		
GNAPHOSIDAE															
**Taieria erebus* Koch L.	15												2		
**Anzacia gemmea* Dalmas					1										
LINYPHIDAE															
Linyphiidae RTU1							4					1			
Mynogleninae RTU1	6	1	1	21	31	37				30		1	38		18
#*Diplocephalus cristatus*							3						4	1	
#*Lepthyphantes tenuis*							12	6		2			6		
#*Microtenonyx subitaneus*	12							1				1	1		
LYCOSIDAE															
**Lycosa hilaris*	287	20	3	11	24	9	9	1		33		4	86	74	3
PSECHRIDAE															
**Poaka graminicola* Forster & Wilton				5	14										
SALTICIDAE															
Holoplatys sp						1									
STIPHIIDAE															
**Cambridgea* n. sp.	4				20								3	1	
THERIDIIDAE															
Dipoena sp.	1														

DOYLE'S BLOCK	DEC/JAN 1998-1999			MAR/APR 1999			JUN/JUL 1999			SEPT/OCT 1999			DEC/JAN 1999-2000		
ORDER Sub Order FAMILY *Species*	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter
COLEOPTERA															
larvae		2	3			3			5				4		
ANTHICIDAE															
#*Trichananca fulgida* Blackburn						1									
SCARABAEIDAE															
#*Aphodius tasmaniae* Hope 1846	4	1			1									1	
CARABIDAE															
#*Laemostenus complanatus* Dejean						2							18		
**Megadromus antarcticus* Chaudoir	5			6						6			1	1	
**Metaglymma moniliferum* Bates	3												2	1	
#*Pentagonica vitipennis*		1													
CERAMBYCIDAE															
#*Arhopalus tristis* Fabricus														1	
**Adriopea* sp.	22	1	12	9		7	13			17			23	2	2
Xyloteles costipennis							1								
Somatidia sp.											1				
COCCINELLIDAE															
#*Coccinella undecimpunctata*		1													
ZOPHERIDAE (COLYDIIDAE)															
Zopheridae RTU1													1		
**Pristoderus antarcticus* White	1						1			1			1		
**Pycnomerus sophorae*	2	2													
CORTICARIDAE															
Corticaridae RTU1	5						4						1		
Corticaridae RTU2													1		
CURCULIONIDAE															
Curculionidae RTUI	2									5			4	1	
Pentarthrum sp. Wollaston													3	1	

DOYLE'S BLOCK	DEC/JAN 1998-1999			MAR/APR 1999			JUN/JUL 1999			SEPT/OCT 1999			DEC/JAN 1999-2000		
ORDER Sub Order FAMILY *Species*	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter
#*Otiorhyncus ovatus* Linnaeus	5	1	12	1		6	1	5		3		4	15		2
#*Listronotus bonariensis* Kuschel								2							
#*Steriphus diversipes* Pascoe	2				1								1		
#*Hylastes ater* Paykull	1						2			2			1		
#*Hylurges ligniperda*	1						7								
ELATERIDE															
#*Agrypnus variabilis* Candeze		1								4			2		
#*Conoderus exsul* Sharp										1	1		4	1	
HISTERIDAE															
**Odontria varicolorata* Given	4	1			1									1	
NITIDULIDAE															
**Epuraea* sp.						1									
OEDEMERIDAE															
**Selenopalpus aciphyllae* Broun 1886	1												1		
PTILIIDAE															
#*Acrotrichus fascicularis* Herbst 1793	2	3						4							3
STAPHYLINIDAE															
Aleocharinae	4					2						2		1	19
Oxytelinae	7	4	4	30	7	4	19		66			6	37	2	7
Staphylininae	1	8	1					1					7	2	
COLLEMBOLA	440	300	100	600	400	550	60	20	20	230	80	320	420	250	300
MYRIAPODA															
Diplopoda															
DALODESMIDAE															
**Icosidesmus variegatus* Carl 1902	1						1	1					7		
SCHEDOTRIGONIDAE															
**Schedotrigona* sp.	1							3			2				36
JULIDAE															

DOYLE'S BLOCK	DEC/JAN 1998-1999			MAR/APR 1999			JUN/JUL 1999			SEPT/OCT 1999			DEC/JAN 1999-2000		
ORDER Sub Order FAMILY *Species*	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter
#*Ophyiulus pilosus* Newport 1842						1									
DERMAPTERA															
FORFICULIDAE															
#*Forficula auricularia*		312			1								50		
DIPTERA															
SCIARIDAE															
larvae						46	162					94			
Sylvicola sp.		2		1										1	
ANTHOMYIIDAE															
#*Anthomyia punctipennis*		1		3										2	
ASILIIDAE															
**Sarapogon* sp.		8			2						2		4	3	
CALLIPHORIDAE															
#*Lucilia sericata*	5	8		3									2	7	
CECIDOMYIIDAE															
Cecidomyiidae RTU1		120		17									15	28	
CHLOROPIDAE															
**Gaurax* sp.		2													
DOLICHOPODIDAE															
Micropygus sp.	14	53	7		8						7		4	32	
DROSOPHILIDAE															
Drosophila sp.		4													
EPHYDRIDAE															
**Psilopa huttoni* Hendel		3													
MYCETOPHILIDAE															
**Mycetophila* sp.	29	250	7	14	130		2				28		17	200	3
STRATIOMYIIDAE															
Odontomyia sp.		6	1	2									8		

DOYLE'S BLOCK	DEC/JAN 1998-1999			MAR/APR 1999			JUN/JUL 1999			SEPT/OCT 1999			DEC/JAN 1999-2000		
ORDER															
Sub Order															
FAMILY	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter	pitfall	pan	litter
Species															
TIPULIDAE															
larvae	1								3	2		15	1		4
**Leptotarsus tapleyi*					2										
**Leptotarsus dicroithorax*	1	15												12	
**Leptotarsus* RTU1.		2			9									1	
**Leptotarsus zeylandiae*				1	17										
HYMENOPTERA															
FORMICIDAE															
**Priocnemis* sp.		2	5												
ISOPODA															
PORCELLIONIDAE															
#Porcellio scaber Latreille		450	359	16	20		1	9		45			78	27	
ORTHOPTERA															
GRYLLIDAE															
**Bobilla* sp.					88		7						4		
RHAPHIDOPHORIDAE															
**Pleioplectron simplex* Hutton	71			28	1		3			8			39	12	
TETTIGONIDAE															
**Conocephalus bilineatus Erichson* 1842					1										

2. Survey 2: Post-treatment Inventory

2.1 The arthropod assemblage: families, taxa and individuals

The 4-week late summer pitfall sample at HR accounted for a total of 3902 individuals from 49 species representing 30 families. At DB, 2852 individuals were trapped accounting for 48 species representing 28 families. There were no additional species trapped which had not previously been accounted for in the characterization survey. A summary of the mean abundance of families, species and individuals in each of the biosolids-treated plot types at both HR and DB is shown in Table 3.5. Note this summary includes collembolan individuals.

Table 3.5. Summary of invertebrate abundance for Hunter's Road and Doyle's Block at family, taxa and individual levels for the post-treatment survey. Plots were treated in September 2000 and sampled in February 2001. Data are the total and mean (±SD) values from 1 pitfall in each of the 3 replicate plots for each of the 3 biosolids treatments.

HUNTER'S ROAD						
		Nil		**400 kg N/ha**		**800 kg N/ha**
Abundance	Total	Mean (±SD)	Total	Mean (±SD)	Total	Mean (±SD)
Families	21	10.3 (1.5)	24	14.3 (3.2)	20	12 (1)
Taxa	31	13 (1.7)	35	17.3 (6.6)	31	13.6 (2.1)
Individuals	370	127 (95.1)	3304	1117.6 (1409.5)	228	83.3 (22.2)
DOYLE'S BLOCK						
Abundance	Total	Mean (±SD)	Total	Mean (±SD)	Total	Mean (±SD)
Families	19	13.3 (0.6)	23	13.3 (4.2)	23	14.2 (2.9)
Taxa	30	17.6 (2.3)	35	18.3 (3.5)	29	18 (2)
Individuals	398	142.6 (37.6)	1694	590 (548.4)	760	265 (260.9)

This data suggests that the moderate rate of biosolids (400 kg N/ha) is having a positive effect on the total number of individuals at both sites. A stepwise analysis of transformed data was performed to identify a biosolids-mediated effect on the abundance of families, taxa and individuals at both HR (Table 3.6) and DB (Table 3.7).

Table 3.6. ANOVA Summary statistics for the abundance of arthropod individuals, taxa and families in plots at Hunter's Road from the post treatment survey. Mean values calculated from transformed data representing all arthropods (including Collembola) trapped by pitfalls during 4 weeks where N = 3 for each of the biosolids treatment rates (Nil, 400 and 800 kg N/ha).

HUNTER'S ROAD					
INDIVIDUALS	**SS**	**DF**	**MS**	P = 0.47	F = 0.85
Treatment	399.7	2	199.9		
Residual	1408	6	234.7		
Total	1808	8			
TAXA	**SS**	**DF**	**MS**	P = 0.44	F =0.95
Treatment	0.45	2	0.22		
Residual	1.42	6	0.24		
Total	1.87	8			
FAMILIES	**SS**	**DF**	**MS**	P = 0.13	F =2.86
Treatment	0.46	2	0.23		
Residual	0.48	6	0.08		
Total	0.94	8			

Table 3.7 ANOVA Summary statistics for the abundance of arthropod individuals, taxa and families in plots at Doyle's Block from the post treatment survey. Mean values calculated from transformed data representing all arthropods (including Collembola) trapped by pitfalls during 4 weeks where N = 3 for each of the biosolids treatment rates (Nil, 400 and 800 kg N/ha).

DOYLE'S BLOCK					
INDIVIDUALS	**SS**	**DF**	**MS**	P =0.5	F =0.77
Treatment	137.1	2	68.55		
Residual	531.1	6	88.51		
Total	668.2	8			
TAXA	**SS**	**DF**	**MS**	P =0.42	F =0.99
Treatment	1.55	2	0.78		
Residual	7.71	6	0.78		
Total	6.26	8			
FAMILIES	**SS**	**DF**	**MS**	P = 0.67	F =0.43
Treatment	0.10	2	0.52		
Residual	0.71	6	0.12		
Total	0.81	8			

There was no evidence to support a biosolids-mediated effect on the abundance of taxonomic units at either family, taxa or individual level at HR (Table 3.6) or DB (Table 3.7). To identify whether the probable under representation of Collembola was confounding the outcome, further analysis at the individual level was undertaken using (i) collembolan counts only and (ii) Arthropods less collembolan counts (Table 3.8). No significant effect was found for either analysis.

Table 3.8 ANOVA Summary statistics for the abundance of collembolan individuals only and arthropod individuals (less collembola) in plots at Hunter's Road and Doyle's Block from the post treatment survey. Mean values calculated from transformed data representing individuals trapped by pitfalls during 4 weeks where N = 3 for each of the biosolids treatment rates (Nil, 400 and 800 kg N/ha).

HUNTER'S ROAD					
Collembola	**SS**	**DF**	**MS**	P = 0.59	F = 0.57
Treatment	363.4	2	1817		
Residual	1906	6	317.6		
Total	2269	8			
Arthropods (less Collembola)	**SS**	**DF**	**MS**	P = 0.45	F =0.92
Treatment	5.53	2	2.77		
Residual	17.96	6	2.99		
Total	23.5	8			
DOYLE'S BLOCK					
Collembola	**SS**	**DF**	**MS**	P = 0.39	F = 1.11
Treatment	304.2	2	152.1		
Residual	819.5	6	136.6		
Total	1124	8			
Arthropods (less Collembola)	**SS**	**DF**	**MS**	P = 0.96	F =0.04
Treatment	0.36	2	0.18		
Residual	26.47	6	4.41		
Total	26.83	8			

2.2 Arthropod abundance in functional groups

There was evidence of a weakly significant effect of biosolids on the proportional abundance of non-feeding individuals at HR (ANOVA, $P < 0.05$, $F = 5.93$) (Table 3.9). Given that few units in this functional group were trapped and the calculated standard deviation, this result was not considered to be strong evidence of a biosolids-mediated effect. There was no other evidence of a biosolids-mediated effect on either the abundance of individuals in functional groups or the abundance of taxa in functional groups at either HR (Table 3.9) or DB (Table 3.10).

Table 3.9 Summary of the abundance of individuals and taxa in functional groups in plots at Hunter's Road from the post treatment survey. Values are the mean proportional abundance of individuals trapped by pitfalls during 4 weeks where N = 3 for each of the biosolids treatment rates (Nil, 400 and 800 kg N/ha). *significant at $P < 0.05$ after stepwise ANOVA of arcsine transformed data.

Hunter's Road					
Individuals	**Nil**	**400 kg N/ha**	**800 kg N/ha**	**P value**	**F**
Predatory	67.54 ±17.96	39.01±26.98	61.54 ±20.93	0.81	0.32
Phytophagous	16.56±18.98	15.05±7.67	13.07±10.89	0.93	0.08
Scavenger	20.64±15.45	20.25±12.46	15.08±9.83	0.91	0.09
Omnivorous	11.56±14.07	2.27±3.93	5.7±8.2	0.8	0.23
Non-feeding	1.93±1.67	0	2.45±0.99	*0.04	5.93
Mycetophagous	1.52±1.45	0.75±1.31	2.12±3.68	0.83	0.18
Taxa					
Predatory	51.1±8.55	45.07±2.25	61.54±20.93	0.34	1.29
Phytophagous	18.88±13.47	27.79±16.27	15.88±10.11	0.65	0.46
Scavenger	7.77±0.96	20.46±19.31	15.08±8.11	0.19	2.19
Omnivorous	7.22±6.73	3.33±5.77	5.71±8.11	0.76	0.28
Non-feeding	9.99±8.81	0	2.45±0.99	0.09	3.54
Mycetophagous	4.99±4.41	0	2.12±3.68	0.28	1.56

Table 3.10 Summary of the abundance of individuals and taxa in functional groups in plots at Doyle's Block from the post treatment survey. Values are the mean proportional abundance of individuals trapped by pitfalls during 4 weeks where N = 3 for each of the biosolids treatment rates (Nil, 400 and 800 kg N/ha).

Doyle's Block					
Individuals	**Nil**	**400 kg N/ha**	**800 kg N/ha**	**P value**	**F**
Predatory	69.51±10.4	61.65±14.96	48.92±15.38	0.22	1.97
Phytophagous	20.61±8.11	27.08±4.26	13.94±5.58	0.33	1.35
Scavenger	0.22±0.38	6.91±11.2	0.5±0.87	0.46	0.89
Omnivorous	2.54±2.65	2.7±4.68	30.59±11.69	0.06	4.62
Non-feeding	2.19±0.82	3.42±3.01	3.79±1.64	0.61	0.54
Mycetophagous	4.91±5.99	3.21±4.8	2.63±3.54	0.76	0.28
Taxa	**Nil**	**400 kg N/ha**	**800 kg N/ha**	**P value**	**F**
Predatory	56.33±5.71	63.27±11.9	5.07±6.1	0.85	0.16
Phytophagous	19.61±7.76	17.56±8.91	19.76±5.36	0.85	0.16
Scavenger	1.85±3.2	1.66±2.88	3.33±2.88	0.74	0.32
Omnivorous	4.23±3.75	1.66±2.88	5.55±0.96	0.29	1.53
Non-feeding	11.96±2.02	12.63±2.69	12.54±2.25	0.95	0.05
Mycetophagous	5.98±71.02	5.83±6.29	5.55±5.09	0.76	0.28

2.3 Arthropod abundance: a ranked summary

A ranked summary of the ten most abundant taxa at HR was dominated by introduced species and included collembola, spiders, earwigs, harvestmen, millipedes and beetle larvae (Table 3.11). The ten most abundant taxa at DB included collembola, spiders, mites, harvestmen, orthopterans and slaters (Table 3.12). More than 50% of the ranked taxa in each assemblage were indigenous.

Table 3.11 The ten most abundant taxa from pitfall traps in untreated plots (Nil) at Hunter's Road approx. 6 months after biosolids application and the comparative catch of those taxa in plots treated at rates of 400 and 800 kg N/ha. Values are the mean (±SD) of 3 replicate plots per treatment. Fx = functional group; P = predatory, H = phytophagous, S = scavenger, O = omnivorous; NZ = indigenous; INT = introduced.

FAMILY Taxon	Fx	Origin	Nil	400 kg N/ha	800 kg N/ha
Collembola	?	?	80 ± 103.9	1005 ± 172.7	29 ± 18.24
Lycosa hilaris	P	NZ	6.33 ± 8.25	26 ±18.45	7.66 ± 5.43
Forficula auricularia	S	INT	5.33 ±7.54	15.6 ± 13.52	4.33 ± 3.68
Lepthyphantes tenuis	P	INT	5 ±3.55	3.33 ±4.74	Nil
Phalangium opilio	P	INT	4.66 ± 6.59	9.66 ± 6.84	8.66 ± 7.36
Coleopteran larvae (non predatory units)	H	?	4 ± 5.63	Nil	Nil
Cylindroiulus britannicus	S	INT	3.66 ± 5.18	Nil	Nil
Nuncia sp	P	NZ	3 ± 4.24	8.66 ± 12.25	12 ± 16.97
Diplocephalus cristatus	P	INT	2.66 ± 2.35	4.66 ± 4.64	3 ± 3.74
Dysdera crocatus	P	INT	1.66 ± 1.69	2.66 ± 3.09	1.33 ± 1.24

Table 3.12 The ten most abundant taxa from pitfall traps in untreated plots (Nil) at Doyle's Block approx. 6 months after biosolids application and the comparative catch of those taxa in plots treated at rates of 400 and 800 kg N/ha. Values are the mean (±SD) of 3 replicate plots per treatment. Fx = functional group; P = predatory, H = phytophagous, S = scavenger, O = omnivorous, NZ = indigenous; INT = introduced.

FAMILY Taxon	Fx	Origin	Nil	400 kg N/ha	800kg N/ha
Collembola	?	?	50 ± 50	500 ±500	180 ± 277.13
Linyphiidae RTU1	P	?	19 ± 25	NIl	5 ± 1.63
Neotrichozetes sp.	P	NZ	16 ± 15.29	18.33 ± 12.12	17.66 ± 10.1
Nuncia sp.	P	NZ	9.33 ± 7.54	3.33 ± 2.86	Nil
Erigone wiltonii	P	INT	4.66 ± 3.39	4 ± 5.65	Nil
Microtenonyx subitaneous	P	INT	3 ± 2.44	Nil	Nil
Phalangium opilio	P	INT	3 ± 4.24	Nil	Nil
Lepthyphnates tenuis	P	INT	3 ± 2.44	2 ± 0.81	2.33 ± 0.47
Pleioplectron simplex	O	NZ	3.33 ± 3.39	Nil	23.6 ± 4.49
Porcellio scaber	S	NZ	3.33 ± 3.39	Nil	Nil

2.4 The trophic structure of the arthropod assemblage

The trophic structure at Hunter's Road and Doyle's Block was characterized based on analyses of the proportional abundance data for (i) individuals and (ii) taxa (after arcsine transformation) for untreated plots only. At Hunter's Road, significant differences in the abundance of individuals in functional groups were identified (ANOVA, $F = 4.55$, $P = 0.05$) (Table 3.13). Predatory

individuals were more abundant than omnivorous, non-feeding and mycetophagous individuals. No difference was found in the proportional abundance of taxa in functional groups in the untreated plots at Hunter's Road (Table 3.14).

Table 3.13 ANOVA Summary statistics of the mean abundance of individuals in functional groups at Hunter's Road for the post-treatment survey.

Source	SS	df	MS	F value	P
Treatment	9.45	5	1.89	4.55	0.015
Residual	4.98	12	0.42		
Total	14.43	17			

Table 3.14 ANOVA Summary statistics of the mean abundance of taxa in functional groups at Hunter's Road for the post-treatment survey.

Source	SS	df	MS	F value	P
Treatment	4.98	5	0.99	4.55	0.024
Residual	7.57	12	0.63		
Total	12.56	17			

At Doyle's Block, highly significant differences in the abundance of individuals in functional groups in the untreated plots were identified (ANOVA, $F = 11.47$, $P = 0.0003$) (Table 3.15). Predatory individuals were more abundant than scavenger, omnivorous, non-feeding and mycetophagous individuals. Phytophagous individuals were more abundant than scavengers. The proportional abundance of taxa in functional groups in the untreated plots at Doyle's Block also highlighted significant differences (ANOVA, $F = 6.01$, $P = 0.005$) (Table 3.16). Predatory taxa were more abundant than scavenger and omnivorous taxa; phytophagous taxa were more abundant than scavenger taxa.

Table 3.15 ANOVA Summary statistics of the mean abundance of individuals in functional groups at Doyle's Block for the post-treatment survey.

Source	SS	df	MS	F value	P
Treatment	12.67	5	2.53	11.47	0.0003
Residual	2.65	12	0.22		
Total	15.32	17			

Table 3.16 ANOVA Summary statistics of the mean abundance of taxa in functional groups at Doyle's Block for the post-treatment survey.

Source	SS	df	MS	F value	P
Treatment	9.09	5	1.82	6.001	0.005
Residual	3.63	12	0.303		
Total	12.72	17			

The apparent dominance, at both sites, of the predatory functional group, is highly likely to have been influenced by the pitfall trapping methodology accounting for a higher proportion of

ground-active, cursorial hunters. The trophic structure at both sites was most clearly defined at the level of individual abundance. However, the high level of variability between the pitfall catches in the three untreated plots, in conjunction with the small sample size restrict generalizations.

2.5 Species diversity (H')

The Shannon-Wiener (H') diversity values for HR and DB were within average values for each plot type, where the normal range for H' varies from 0.5 – 3 (Table 3.17). Biosolids applications were not found to have had a significant effect on species diversity (H') at either HR (ANOVA, F = 0.54, P = 0.61) (Table 3.18) or DB (ANOVA, F = 0.9, P = 0.45) (Table 3.19). Sorenson's Index (Sim.) suggested a low turnover in the species assemblage between plots at both sites, indicating reasonable agreement in the similarity of the assemblage, irrespective of treatment rate or site.

Table 3.17 Summary of the mean Shannon-Wiener (H') diversity value (± SD) and Sorenson's Similarity Index (Sim.) for invertebrate species at Hunter's Road and Doyle's Block in plots receiving Nil, 400 and 800 kg N/ha. Values for H' were derived from the mean abundance of species from pitfalls in 3 replicate plots at each of the three biosolids treatment rates. The values for the Sorenson Index were derived from a presence/absence matrix for each treatment rate.

	Hunter's Road	**Doyle's Block**
Treatment rate (kg N/ha)	Shannon-Wiener (H')	Shannon-Wiener (H')
Nil	1.0 ± (1.04)	2.32 (±0.89)
400	1.73 (± 1.35)	1.32 (± 0.91)
800	1.78 (± 0.52)	1.71 (±0.95)
Treatment pairing	Sorenson Index	Sorenson Index
Nil/400	0.64	0.64
Nil/800	0.61	0.64
400/800	0.69	0.68

Table 3.18 ANOVA Summary statistics for Shannon-Wiener diversity (H') at Hunter's Road for the post-treatment survey.

Source	SS	df	MS	F value	P
Treatment	1.141	2	0.571	0.54	0.61
Residual	6.365	6	1.06		
Total	7.506	8			

Table 3.19 ANOVA Summary statistics for Shannon-Wiener diversity (H') at Doyle's Block for the post-treatment survey.

Source	SS	df	MS	F value	P
Treatment	1.535	2	0.763	0.9	0.45
Residual	5.07	6	0.845		
Total	6.59	8			

2.5 Indicator Species Analysis

An arbitrarily selected cut-off point of 50% for an Indicator Value was utilized for the Indicator Species analysis to help reduce noise from the dataset. At HR (Table 3.20) the introduced, predatory spiders *Lepthyphantes tenuis* and *Dysdera crocata* and predatory staphylinid beetles from the family Aleocharinae most typical of untreated plots. Several taxa were strongly indicative of treated plots. These included the introduced, predatory harvestman *Phalangium opilio,* the phytophagous weevil *Otiorhyncus ovatus*, the scavenger earwig *Forficula auricularia* the indigenous wolf spider *Lycosa hilaris,* the introduced spiders *Diplocephalus cristatus* and *Ostearius melanopygus* and the small black cricket *Bobilla* sp.

Table 3.20 Two-way indicator table showing the species indicator power for Hunter's Road plot types (treated or untreated) following hierarchical clustering. An arbitrary cut-off level for the Indicator Value of ≥ 50% was used. The Indicator Value column indicates the value of that species as an indicator for that clustering level. The dichotomy for the first division was based on either untreated (control plots) or treated (plots receiving biosolids at either 400 or 800 kg N/ha). + predatory specimens ^non-predatory specimens.

	HUNTER'S ROAD			
Untreated plots	**IV (%)**	**MEAN**	**SD**	**P VALUE**
Lepthyphantes tenuis	100	40.5	15.84	0.014
Dysdera crocata	86.2	55.9	13.72	0.076
Aleocharinae spp.	83.3	43.9	13.83	0.046
Nuncia sp.	75	34.6	15.46	0.048
Coleopteran larvae ^	75	35	15.86	0.048
Erigone wiltonii	75	34.5	14.95	0.047
Coleopteran larvae +	50	27.3	15.25	0.171
Neotrichozetes RTU1	50	28.2	13.84	0.161
Treated plots	**IV (%)**	**MEAN**	**SD**	**P VALUE**
Phalangium opilio	100	44.2	14.84	0.014
Otiorhyncus ovatus	100	45.7	16.24	0.014
Forficula auricularia	100	46.5	15.34	0.014
Lycosa hilaris	99	51.3	14.76	0.014
Diplocephalus cristatus	95	51.4	15.12	0.014
Ostearius melanopygus	80	39.5	14.75	0.046
Bobilla sp.	80	40.9	15.99	0.046
Collembola	71.7	84.8	11.67	0.833
Hypharpax australis	60	36.6	15.55	0.161
Laemostenus complanatus	60	36	15.15	0.154

At DB (Table 3.21) unidentified linyphid spiders and staphylinid beetles from the family Aleocharinae were most typical of untreated plots. In treated plots, both the spider *Erigone wiltonii* and the harvestman *P. opilio* were typical.

Table 3.21 Two-way indicator table showing the species indicator power for Doyle's Block plot types (treated or untreated) following hierarchical clustering. An arbitrary cut-off level for the Indicator Value of ≥ 50% was used. The Indicator Value column indicates the value of that species as an indicator for that clustering level. The dichotomy for the first division was based on either untreated (control plots) or treated (plots receiving biosolids at either 400 or 800 kg N/ha). + non-predatory specimens.

DOYLE'S BLOCK				
Untreated plots	**IV (%)**	**MEAN**	**SD**	**P VALUE**
Linyphiidae RTU2	100	55.1	16.07	0.013
Linyphiidae RTU1	84.2	55	13	0.042
Aleocharinae RTU1	83.3	49.1	16.19	0.078
Lycosa hilaris	74.6	54.5	15.57	0.203
Pleioplectron simplex	69.7	51.5	15.31	0.189
Adriopea RTU1	62.5	61.2	7.72	0.419
Treated plots	**IV (%)**	**MEAN**	**SD**	**P VALUE**
Erigone wiltonii	100	36.1	17.41	0.013
Phalangium opilio	100	36.8	17.04	0.013
Odontria varicolorata	66.7	30.6	12.3	0.087
Microtenonyx subitaneus	66.7	30.4	12.9	0.087
Selenopalpus aciphylae	63.6	37.3	15.38	0.087
Neotrichozetes RTU1	63.2	58	10.38	0.323
Coleopteran larvae +	53.3	33.7	19.4	0.236
Nuncia sp.	52.6	64.6	13.52	0.737

2.6 DCA Ordination

The DCA ordinations relating to the mean abundance of invertebrate species at HR (Figure 3.3) and DB (Figure 3.4) in relation to their occurrence in plot treatments indicated species were intermixed, with substantial variation in their spatial relationship. No consistent difference was found to account for the effect of biosolids treatment on species abundance.

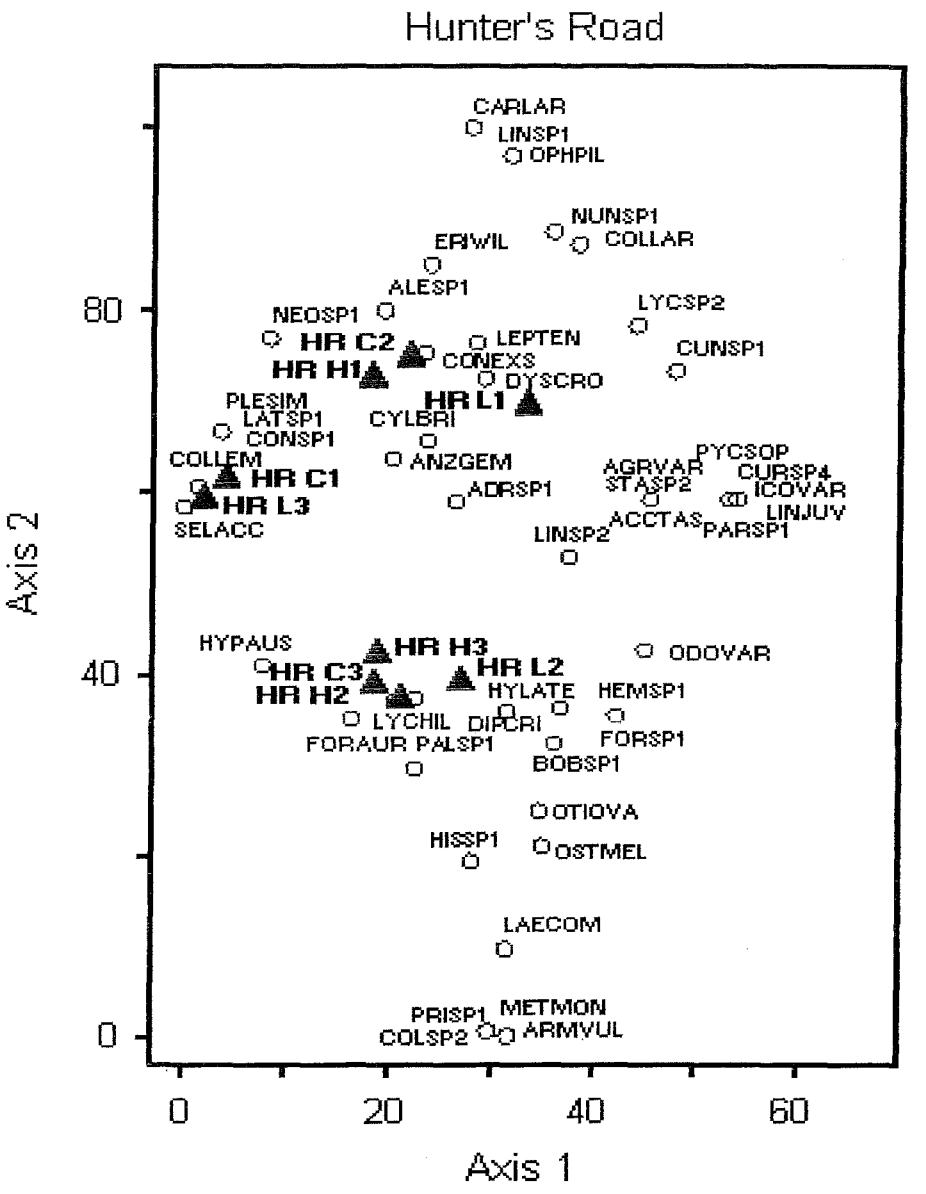

Figure 3.3. DCA scatter plot for the ordination of arthropod taxa trapped by pitfalls at Hunter's Road in replicate plots treated with biosolids at rates of Nil (C), 400 kg N/ha (L) and 800 kg N/ha (H). The replicate treatment plots are marked ▲ and species as ○. The scale marks are in units of the average standard deviation of species turnover. Full taxonomic names are given in the Appendix Table B1.

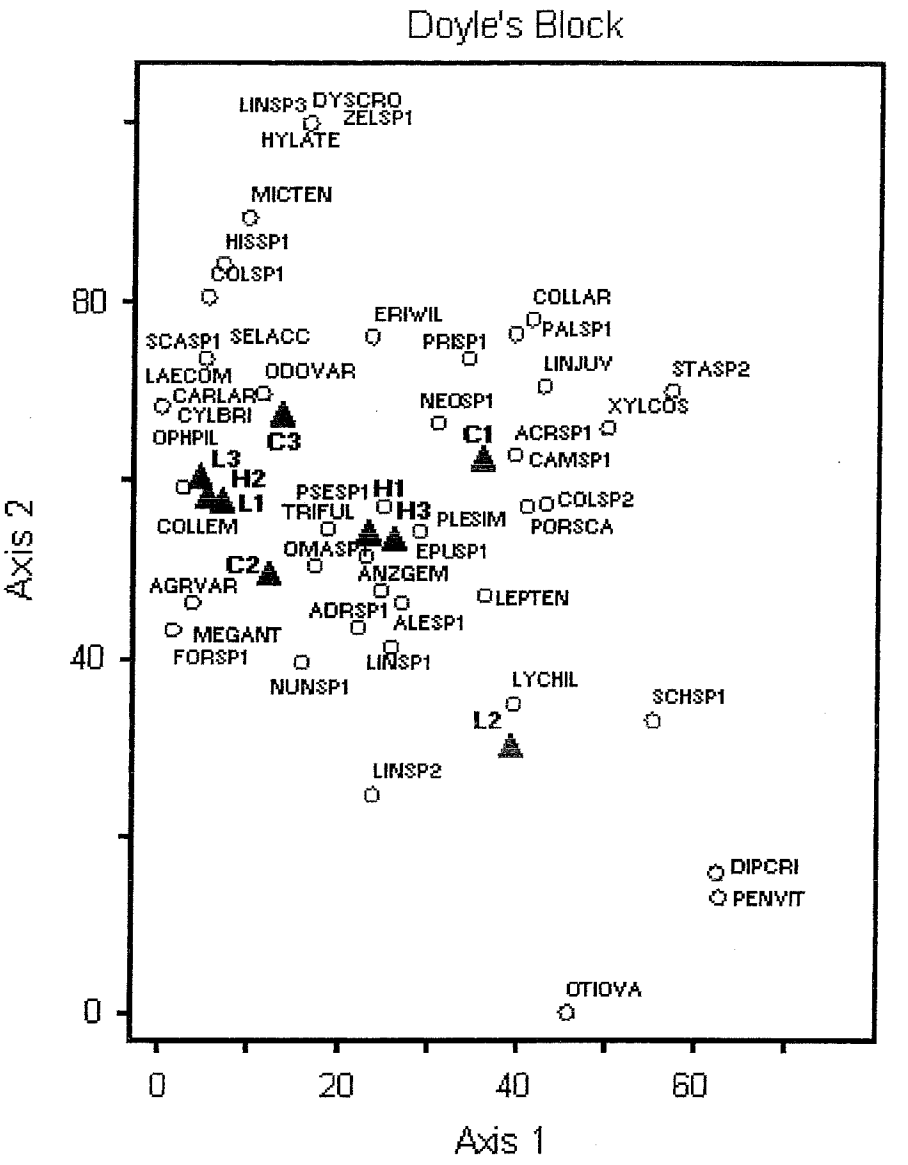

Figure 3.4. DCA scatter plot for the ordination of arthropod taxa trapped by pitfalls at Doyle's Block in replicate plots treated with biosolids at rates of Nil (C), 400 kg N/ha (L) and 800 kg N/ha (H). The replicate treatment plots are marked ▲ and species as ○. The scale marks are in units of the average standard deviation of species turnover. Full taxonomic names are given in Appendix TableB1.

D. DISCUSSION

The diversity of the arthropod assemblage associated with the soil and litter under P. radiata

The taxonomic inventory defines, characterizes, complements and extends the current understanding of the association of soil and litter invertebrates with exotic *P. radiata* planted forests in New Zealand. As an entity in their own right, these monocultures cannot fairly be deemed "ecological deserts" because there is no lack of diversity in the invertebrate assemblage.

Mean diversity (H') values of 1.0 ± 1.04 in untreated plots at HR and 2.32 ± 0.89 in similar plots at DB compare very favourably with normal diversity (H') values which vary from 0.5 – 3 (Gotelli and Colwell 2001). Furthermore, diversity in the biosolids amended plots also compares favourably with Gotelli and Colwell's range. For these reasons, it is contended that the report developed for the CCC was incorrect in stating that "the forests lack diversity"(Anon. 1996).

The planted forest as reservoir for local biodiversity

The landscape surrounding the mid Canterbury planted forests is highly modified pastoral grazing, cropping and intensive dairying, with few unmanaged areas. This limits the value of the surrounding landscape as a source of colonizing populations of mobile indigenous species (particularly beetles) (Harris and Burns 2000) and increases the value of the planted forest as a biodiversity reservoir. It is possible that local biodiversity is maintained and even stimulated by frequent disturbance to the forests (e.g. pruning, harvesting, windrowing, weed spraying). This is because a patchwork of compartments in close proximity and at different stages of development, provide a series of habitats at varying levels of complexity offering different microclimates (Hawke and Wedderburn 1994).

The planted forests may be providing refuge for some indigenous species endemic to the Canterbury region. One example is the spider *Taieria erebus,* which was present at both HR and DB. Johns (1986) noted this species to be "present as far as known only in Christchurch and the Port Hills" and Mcfarlane *et al.* (1999) recorded it as being "generally confined to wooded areas in the Christchurch and Banks Peninsula districts". Similarly, the loess burrowing weta, *Hemiandrus* sp., was trapped occasionally at HR, having previously only been recorded from Banks Peninsula, urban Christchurch, and also one other small population in South Canterbury (P.M. Johns, pers.com.).

Microclimate and its effect on the species assemblage

Both microclimate variability and the effects of seasonality within the species' assemblage were pronounced in the younger stand, HR, compared with that of the mature stand, DB. For example, the winter pitfall tally at HR of 88 individuals from 12 families, was substantially lower than at DB, where 373 individuals representing 21 families were trapped. This suggests DB provided a more seasonally stable microclimate favouring year-round invertebrate activity or supporting populations with multiple generations within a year. It was expected that incoming radiation (affecting the available light and temperature), precipitation (affecting forest floor moisture) and canopy formation (affecting the understorey vegetation) are likely to have contributed to these

observed fluctuations. Previous studies have identified microclimate as a key determinant of invertebrate activity in forests (McColl 1974a).

The proportional representation of indigenous and introduced species

Despite the profound differences in the abiotic character of HR and DB, there was a high level of similarity (Sim.) in the species assemblage, with at least 50% of species shared between each of the two sites. The proportional representation of indigenous and introduced species for the orders Araneae, Myriapoda, and Diptera differed between sites, however no clear trends were identified.

Indigenous beetles

The habitat supports a surprisingly wide variety of indigenous coleopterans, given their distance from native vegetation. However, beetle abundance was highly variable. For example, the cerambycid *Adriopea* sp. was generally abundant throughout the seasons at both HR and DB, in contrast to the single nitidulid *Epuraea* sp. taken from DB in summer. Many of the presumably less tolerant coleopterans were represented by singletons or only a few individuals. An example is the oedemerid beetle *Selenopalpus aciphyllae,* which is otherwise common throughout the eastern side of the South Island and generally associated with flowers of various native trees and shrubs It is interesting to note that this oedemerid constituted a substantial fraction of the malaise trap catch in a study conducted at the Eyrewell Forest pine plantations in North Canterbury (E. Brockerhoff pers. comm.). This difference points to one of the inherent difficulties in interpreting species representation where different methodologies are employed.

Other specimens only occasionally trapped included the carabids *Metaglymma moniliferum* and *Megadromus antarcticus* and the zopherids *Pycnomerus sophorae* and *Pristoderus antarcticus.* Johns *et al.* (1980) took the related subcortical feeders *Pristoderus* sp. and *Pycnomerus* sp. from under the bark of *Pinus nigra,* noting their very specific preference for dead timber.

It is probable that a lack of nearby founder populations and the absence of specific resources necessary for juvenile development are contributing factors which limit the proportional representation of indigenous beetles to less than 40%. This interpretation is based on a recent North Island study, which noted 61% of the malaise-trapped beetle species resident or dispersing through modified pastures near kahikatea (*Dacrycarpus dacrydiodes*) fragments to be indigenous (Harris and Burns 2000). However, the mid Canterbury survey differed in that ground-active rather than flying species were targeted. In order to better validate the observed difference, a mid

to late summer malaise-trapping survey at HR and DB could be useful, but only if trapped species were able to be definitively assigned to have associations with the litter and soil habitat.

Indigenous spiders

The fraction of the indigenous spider biota associated with the mid Canterbury forests represents a combination of highly tolerant species, opportunistic generalists who do not appear to be restricted in their resource requirements and species with marginal populations. Arachnid examples which appear to be tolerant include the psechrid *Poaka graminicola*, which was trapped at both HR and DB, and has previously been recorded from open country, forest margins, grasslands and low plants (Forster 1967, Macfarlane *et al.* 1999).

The percentage of indigenous spiders was higher at HR (57%) than at DB (42%), although the total abundance of all arachnid individuals at either site was almost identical. The physical character of the stand architecture at HR may have provided more available niches for utilization by the spiders. For example, the small lynx spider *Oxyopes gracilipes* was found occasionally, and only at HR. The lynx spiders hunt by running and jumping and are usually found in association with low grasses and shrubs. *O. gracilipes* is a native species, shared with Australia (Ward *et al.* 1999).

Marginal indigenous populations in the planted forests

Some indigenous species which were only rarely trapped at either HR or DB, may be maintaining very marginal populations in these forests. For example, *Lycosa* n.sp. had previously been thought to be restricted to dry riverbeds in the Canterbury area (C.Vink, pers. comm.), but was found at HR. By contrast, the closely related *Lycosa hilaris* was very abundant at both HR and DB, and is commonly observed in home gardens and paddocks (Forster 1967).

Introduced species

As expected, many of the invertebrate species recorded were introduced, ground-active, behaviourally-plastic species, tolerant of disturbance and tending to have generalist resource requirements. A typical coleopteran example is *Laemostenus complanatus*. This introduced European beetle, present at both HR and DB, is common in home gardens, pasture and undisturbed woody vegetation (Johns 1986). Similarly, the colourful spider *Dysdera crocata* which favours open spaces was commonly trapped at HR and has a worldwide distribution.

Orders contributing to functional diversity

The functional diversity of the invertebrate assemblage was greatly enhanced at both sites by the representation of the Myriapoda. This group is of interest because it consists of a small number of both introduced and indigenous scavengers, fungivores and predators. The millipedes are important in comminuting dead plant material, whilst their frass constitutes a resource for microbes and micro-arthropods. Many of the Myriapoda introduced in the nineteenth century are well established and have probably displaced indigenous species in disturbed habitats (Johns 1962).

Introduced millipedes present included *Ophyiulus pilosus* (HR, DB) and *Cylindroiulus britannicus* (HR), which have commonly been reported throughout gardens, agricultural land, native forests and grasslands (Johns 1962). The endemic millipede, *Icosidesmus variegatus* was present at HR, whilst *Schedotrigona* sp. were prevalent in litter in summer at DB and a singleton was taken by pitfall at HR. There are at least five endemic species known from the family Schedotrigonidae and many more are undescribed (Ward *et al.* 1999). Specimens from this family have been taken from *Pinus nigra, P. radiata* and *Nothofagus* forest near Hanmer The archaic chilopod *Lamyctes emarginatus* was trapped only at HR in the warmer months. *L. emarginatus* is commonly found in scrub and forest, household gardens and modified habitats. This chilopod burrows through the soil in search of insect eggs and is likely to be instrumental in aeration of the upper soil and litter (Johns 1986).

Accumulated litter as a habitat for the biota

Stand architecture and the stage of development of a stand not only influences the microclimate of the forest floor, but also the composition of the forest floor. In stands with a developed canopy, the accumulated needle litter develops a characteristic complexity of structure with time. Newly fallen material accumulates at the surface and recalcitrant needle components are progressively integrated in the mycelia of the litter and soil fungi. Baseline microbial and bacterial populations involved in the decomposition of the needle litter provide a food resource for successive trophic levels (Hasegawa and Takeda 1996, Berg *et al.* 1998, Yeates and Saggar 1998).

Litter samples were generally dominated by Collembola, which are known to include mycetophagous, microbivorous and scavenging species (Greenslade 1996, Pawert *et al.* 1996). They were highly abundant and active in the warmer months at DB, although populations are likely to have been restricted by the exposed conditions of summer at HR. This supports observations in West Coast forests of New Zealand where populations are typically depressed

during the hot, dry months (McColl 1974a). The observed reduction in collembolan winter activity at HR and DB could be attributed to predation, reduced availability of preferred food resources, or reduced breeding success due to the nutrient status of the preferred diet (Greenslade 1996).

The litter as a habitat for indigenous dipterans

The accumulated litter at DB also provided habitat for the juvenile stages of the four indigenous tipulid species *Leptotarsus tapleyi, L. dicroithorax, L. zeylandiae* and *Leptotarsus* RTU1. Adult stages were trapped in pitfalls at the two peak periods of emergence, early summer and late summer. Juvenile stages, were observed to be numerous at the litter/mineral soil interface but were numerically underrepresented in the survey. This is because the larvae are slow moving, prone to desiccation and were also probably restricted by the size of the mesh used in the Berlese-Tullgren apparatus.

These tipulid larvae are myceto/geophagic, unlike other closely related, phytophagous, pest species (Coulson 1959, Rief 1996). Given that the earthworm fauna is virtually absent in these mid Canterbury forests (pers. observation), indigenous tipulid larvae may provide compensatory ecosystem services through the translocation of fungal inoculants through the mineral soil (Perel *et al.* 1971) and by physically altering the structure of the soil (Lavelle *et al.* 1997). Such ecosystem processes are important at the microsite scale (Giller 1996). At that scale, activities which might compromise the success of tipulid populations may compromise ecosystem function.

The under storey vegetation enhances biodiversity

During summer, flowering of the under storey woody weeds gorse and broom attracted the honey bee *Apis mellifera* to HR. The phytophagous oedemerid *Selenoplapus aciphyllae*, possibly attracted by flowering plants, was encountered in low abundance in the warmer months at HR and only rarely at DB. The understorey vegetation at HR also provided anchor points for web attachment by the cobweb spider *Achaeranea verruculata*. This spider, commonly distributed throughout the southern South Island, builds a criss-cross web of irregular threads on low level shrubs and prefers settled sites, preying on flies and ants (Forster 1967, Forster *et al.* 1988).

The value of rotting wood as an invertebrate resource

Several of the indigenous species taken from HR and DB had previously been recorded as having associations with exotic planted forests in New Zealand. Johns (1980) noted the orthopteran *Pleioplectorn simplex* was almost always found in the large spaces within or under rotten logs in

Pinus forests. At DB, wind thrown trees, waste prunings and the residual decaying wood from the previous rotation are likely to have provided habitat. The species was not found at HR, although decaying logs in adjacent windrows may support some populations. These predatory, jumping cave wetas may occupy and hunt territorially in the planted forest. Mcfarlane *et al.*(1997) also recorded *P. simplex* from a survey of pine shelterbelts near Christchurch and Johns (1986) noted them present on Banks Peninsula.

Relating the Canterbury study to other east coast surveys

The mid Canterbury survey presented differs substantially in methodology, intent, depth and scope from two other reliable invertebrate biodiversity surveys of planted forests on the east coast of the South Island (Johns *et al.* 1980, Macfarlane *et al.* 1999). Key differences were in the recording of the absolute abundance of species and their seasonal occurrence and the use of permanent plots and an easily replicated sampling strategy. Despite these differences, it has been possible to identify the species in common with the other South Island study sites. This serves to reinforce known distributions, adding to the understanding of invertebrate distributions in the region.

The arthropod survey of the Hanmer State Forest prepared by Johns *et al.* (1980) provided one of the most comprehensive checklists of the assemblage present under *P. radiata* and *P. nigra* in a high country location. Eighteen of the species listed in that survey were also found in the lowland mid Canterbury survey. The survey presented by Macfarlane *et al.* (1998) of lowland pine shelterbelts near to Christchurch, had more species in common with the mid Canterbury sites. Twenty nine of the species listed were found to be in common with the mid Canterbury forests.

The effect of biosolids applications on the invertebrate assemblage

The application of biosolids to the *P. radiata* plantations at Hunter's Road and Doyle's Block does not appear to have had a substantial effect on ecosystem processes. It is stressed, however, that this is a short-term viewpoint (Setälä *et al.* 2000). Although there were indications from the data that biosolids treatments at the 400 kg N/ha rate had a positive effect on arthropod abundance, it was shown that this increase was due mostly to the presence of collembolans. Further analysis showed that collembolan abundance was highly variable within treatments. Given that these individuals are known to respond rapidly to positive pulses in nutrient availability, warmth and moisture (Greenslade, 1996) further work at the study sites may profitably be targeted at these arthropods.

The functional group analysis highlighted only one incidence, at HR, of a biosolids-mediated effect. The total absence of non-feeding species in the 400 kg n/ha pitfalls contrasted sharply with their abundance in the 800 kg N/ha. Plots. The non-feeding functional category refers exclusively to the adult craneflies, which are known to be weakly represented at younger stands, such as HR, due mainly to the sparse litter layer inhabited by the larvae. Speculation on the importance of this outcome is restricted by the very few individuals trapped.

The lack of evidence of a biosolids-mediated effect on the arthropod assemblage may be an artefact of the sampling strategy, or the patchy distribution of species in space. Furthermore, simple species richness (the number of species present) data may be either too coarse or too simplistic to adequately monitor changes. A more sensitive methodology capable of identifying fine grain changes at the species-level is to quantify the biomass of individuals.

The lack of an effect at species level was surprising, given that biosolids applications represent a high input nutrient pulse (Brockway *et al.* 1986) that could be expected to alter the existing balance of species because it changes both the energy budget and the physical habitat. Other studies in forest and grassland systems have identified nutrient fluxes as key mechanisms driving community change (Anderson and Ineson 1984, Chen and Wise 1997, Bird *et al.* 2000). One classic example is the nutrient enhancement of grassland systems, which tends to foster microbial growth and favour bacterial pathways of decomposition, in turn supporting opportunistic bacterial-feeding fauna (Bardgett and Cook 1998).

The observed lack of effect under these *P. radiata* stands could have been due to a number of factors. These include (i) delays in the release of nutrients from the biosolids, which are known to be a slow-release fertilizer (Bourke *et al.* 1997); (ii) the immobilization of the nutrients by the fungal and microbial population (Treseder and Allen 2000); (iii) the possibility that after 6 months, much of the nutrient flux had already passed; (iv) a reflection of the capacity of the invertebrate community to respond; (v) the existing community may be insensitive to enrichment and; (vi) the buffering capacity of the soil environment may have masked any effects (Setälä 2002). These unknowns beg for further examination.

Of these possible explanations, the literature tends to support a lack of sensitivity (of both the habitat and the biota within) as a plausible interpretation of no effect. Laboratory and field experiments indicate faunal groups typical of managed forests are buffered against drastic changes to the environment by the soil organic layer (Setälä 2002). A recent simulation model

constructed for carbon and nitrogen transfers among plants and functional groups of microbes and soil fauna, suggested ecosystems could sustain the loss of some functional groups with little decline in ecosystem services because of compensatory changes in the abundance of surviving functional groups (Hunt and Wall 2002). In that study, the model deleted 15 functional groups of microbes and soil fauna one at a time. Only three deletions (bacteria, saprophytic fungi and root-feeding nematodes) caused as much as a10% change in indices of ecosystem function (nitrogen mineralization and primary production). These results may point to the resilience and resistance of the assemblage and its environment to perturbations and may be an inherent characteristic of the soil fauna (Elliott and Lynch 1994). Alternatively, it could indicate a high level of redundancy in the system so that the lack of their contribution to a particular process is compensated by other species.

The trophic structure characterizes functional diversity in the planted forest

Trophic structure was examined in two ways, first in relation to the proportional abundance of individuals in functional groups and second, in relation to the proportional abundance of taxa in functional groups. The trophic structure of both sites was clearly dominated by predatory individuals, whilst mycetophagous, omnivorous and non-feeding individuals occupied the lowest tier. However, the sites differed in relation to the phytophagous and scavenger individuals. At HR, the phytophagous and scavenger groups could be considered to be offset parallel to the top tier; at DB, phytophagous individuals were at least parallel to the top tier predators and clearly dominant over the scavengers.

Although generalizations are drawn with caution (given the sample size and within-plot variability), predator dominance is likely to be the default for the soil and litter assemblage in similar planted forests in the region. The second tier appears to be less well-defined, whilst the third tier is strongly occupied by omnivorous, mycetophagous and non-feeding individuals. The non-feeding functional group (used because of the occurrence of adult craneflies in the pitfall samples) cannot be reasonably ignored because they represent a substantial fraction of the myceto/geophagic soil and litter fauna in the larval stage.

Examined at the level of species (or taxa), a differentiation between trophic levels at HR was not evident. However, at DB, there was a shared dominance by the predatory and phytophagous taxa over scavengers; predatory taxa also dominated the omnivorous taxa. Thus, at species level, the mycetophagous and non-feeding groups only were on the lowest tier.

The value of this analysis lies principally in examining theories relating to biodiversity, particularly functional redundancy (Ehrlich and Ehrlich 1981). Functional redundancy (in terms of individual abundance) appears comparatively high at the peak of the trophic triangle, but relatively low at the base. Thus, the loss of individuals from any of the lowest tier functional categories represents the most serious threat to functional diversity in the planted forest. However, it is a moot point as to whether such losses have a quantifiable impact on ecosystem processes (Hunt and Wall 2002). Compensatory changes in the abundance of surviving groups could balance out losses leading to stabilization. However, these losses could still represent a reduction in local biodiversity.

Functional redundancy and species diversity

An example of the functional redundancy/local biodiversity argument is provided by the myceto/geophagic tipulid larvae. This family is represented by four species at DB (and very occasionally at HR) and are the only species readily classified in that functional group at that site. Although the model proposed by Hunt and Wall (2002) suggests their combined loss is unlikely to have an impact on ecosystem services, any species-level loss would compromise local biodiversity.

Similarly, local biodiversity would be reduced if species losses occurred amongst predatory spiders which are secondary consumers at the peak of the detrital food web (Larsen *et al.* 1994). Although presumed to have no detectable influence on any ecosystem-level processes (Setälä *et al.* 2000) spiders are important components of functional diversity because of their involvement in energy transfer through terrestrial food webs. Differences in spider abundance and diversity which are attributable to biosolids application, may be detected in trophic regulation and could be a valuable area for future investigations. The degree to which predatory pressure either limits lower trophic populations, or whether resource limitations govern higher trophic populations in these forests could be examined in the context of biosolids applications (Chen and Wise 1999). Key indicator species selected from the ISA suite could be suitable species for investigation.

Incremental biosolids applications and species diversity

There was no evidence for a significant effect of biosolids treatment rate on invertebrate diversity (H') at either site. However, as noted previously, interpretation of the response is guarded, given the variability within plots receiving similar treatments and the low level of replication.

The physical effect of the weather on biosolids may influence species-level responses

It is important to note that the observed outcomes from the post treatment survey may have been confounded due to the extremely wet weather which occurred during and immediately after the application trial. Heavy rains were observed to cause the dewatered biosolids to imbibe water, after which they dried to form an extensive thick, soft crust over the forest floor surface. The lack of a canopy at HR meant that evaporation was rapid, resulting in the biosolids having the constitution of thick cardboard; after six months, the woody weeds and grasses had progressively penetrated and separated the material. However, at DB, the biosolids were observed to have retained their blanket coverage (particularly at the 800 kg N/ha rate) and to form a layer between existing and newly fallen litter.

There are many possible effects on the invertebrate population arising from these conditions which could alter species dynamics. These include, for example, mobility, avoidance, attraction, lateral migration and restricted gaseous transfer. In a later chapter, the physical effects of biosolids application on a potentially ecologically relevant species is demonstrated.

DCA analysis

Given the degree of similarity in the arthropod assemblage, it was not surprising that the DCA was unable to separate the biosolids treatment plots according to the rate of application on the basis of the abundance of invertebrate species present. Patterns may have been obscured where within-treatment and between-plot variability was high, caused by, for example, the patchy distribution of species. Patchy distributions are a feature of habitat heterogeneity. At the microhabitat scale, the forest floor may be extremely heterogeneous in both structure and resources for microarthropods. Heterogeneity at that scale has previously been shown to influence the distribution and abundance of small insects and arachnids (Dennis *et al.* 1998).

Indicator species analysis

The ISA provided a limited number of species with a high and significant indicator value suggesting they could be useful as bioindicators of effect in these planted forests. Future invertebrate research at these sites could reliably employ pitfall traps to account for changes in either the species assemblage typical of treated or untreated plots, or variation in the abundance of the indicator species which were not accounted for by normal seasonal fluctuations.

Suites of indicators are recommended

Both indigenous and introduced species, many of them adventive, were found to rank highly as indicators of plot type. However, species representation in plot types between treatments and sites was not always in agreement. For example, the indigenous wolf spider *Lycosa hilaris* was abundant in treated plots at HR, but was also a significant indicator in untreated plots at DB. Similarly the linyphiid *Erigone wiltonii* had good indicator power in untreated plots at HR and the highest ranking in treated plots at DB. Whilst this duality is difficult to explain, it does reinforce the need to adopt a suite of bioindicators for these sites in future studies (McGeoch 1998, Frouz 1999).

Statistically reliable indicators

Both time and expertise is required for the sorting and identification of invertebrates species. The ISA provides not only a short cut for future monitoring, but also statistical confidence in the indicator potential of selected species. However, the value of such a list is limited by the successional development of the forest and the altered suitability of sites to support some species. Thus, a flexible approach is needed.

Future monitoring studies at HR (or similar aged-stands) could reliably examine the abundance of at least *Lepthyphantes tenuis* and *Dysdera crocata* (in untreated plots), and *Phalangium opilio* and *Otiorhyncus ovatus* (in treated plots). DB presents a less easily assessed situation, as the key indicators were members of the Linyphiidae, a notoriously difficult group to diagnose taxonomically (David Blest, pers. com.). Furthermore, the staphylinid beetle family, Aleocharinae, was the next highest ranking group, and whilst simple to identify at that level, species level identification in New Zealand is not. Staphylinids have been advocated as suitable bioindicators in European studies where the taxonomy is more better understood (Bohac 1999) Because of their abundance (both juvenile and adult forms) in disturbed and modified habitats, staphylinids represent a family worthy of closer investigation.

Conclusions

The composition of the invertebrate assemblage under *P. radiata* in lowland mid Canterbury stands appears driven by a combination of seasonal effects, habitat structure and the distinctive abiotic characteristics of *P. radiata* (Gardner *et al.* 1995, Scholes and Nowicki 1998). Despite the highly modified surrounding landscape, a greater than average proportion of the species present in the planted forest are indigenous. Although some were rare in this habitat, it is not known whether any are threatened. It is obvious, however, that each additional species adds to local

CHAPTER FOUR

THE FORESTRY CYCLE, HABITAT HETEROGENEITY AND THE IMPLICATIONS FOR INVERTEBRATE DIVERSITY

A. INTRODUCTION

Planted forests are an ideal model for testing theories relating local terrestrial arthropod diversity to the three dimensional concept of habitat complexity. On a local scale, planted forests may exhibit a level of spatial homogeneity which is age-related (Hutcheson and Jones 1999, Bonham *et al.* 2002) and can largely be accounted for by similar management histories and silviculture regimes (Lewis and Ferguson 1993). At the scale of the individual stand, time-related changes in the vertical and horizontal architecture of the stand may influence the complexity of the planted forest as a habitat. Therefore, the stage of stand development could be expected to influence the degree of observed homogeneity. The intent of this study is to examine whether the diversity of terrestrial arthropods associated with the forest floor adequately reflects the assumption of time-related variability of the spatial characteristics of their habitat.

Spatial heterogeneity refers to the variety of content or complexity within space. The spatial heterogeneity theory predicts a positive relationship between habitat complexity and species diversity (Davidowitz and Rosenzweig 1998) and has found widespread support in a variety of terrestrial habitats (Gardner *et al.* 1995, Walter and Himmler 1996, Hanowski *et al.* 1997). For example, the diversity of small insects and arachnids has been shown to increase asymptotically in relation to vertical and horizontal components of indigenous grassland vegetation structure (Dennis *et al.* 1998). Similarly, the density of orb-weaving spiders in old fields has been shown to increase as the vegetation architecture increases in complexity (McNett and Rypstra 2000).

In planted forest habitats, evidence at the individual stand scale has indicated that increases in the richness and diversity of the biota is attributable to a high level of spatial heterogeneity promoted by variation in the structure of the canopy (Allen *et al.* 1995, Hanowski *et al.* 1997, Ogden *et al.* 1997, Ings and Hartley 1999, Jukes *et al.* 2001). This is because the canopy moderates stand microclimate and the available light for plant growth (Hawke and Wedderburn 1994, Walter and Himmler 1996, Tanabe *et al.* 2001). Microclimate is a key mechanism driving the diurnal and seasonal activity responses of forest floor invertebrates (McColl 1974a, Bird *et al.* 2000) and

could also be expected to moderate the suitability of the forest floor habitat for some invertebrates.

The four predictable and progressive stages relating to stand development of *P. radiata* are arbitrary designations based on stand appearance and will vary between regions, sites, genotype and management options (Lewis and Ferguson 1993). The generalized stages summarised from that text include: (i) the establishment stage (up to 5 years), characterized initially by exposed soils, a broad diurnal temperature range and high levels of evapotranspiration; (ii) the pre-canopy stage (up to approx. 10 years but may extend up to 20 years) during which pruning or waste thinning and woody debris has accumulated on the forest floor; (iii) the post-canopy stage (20+ years onwards), which features progressive accumulation of litter on the forest floor, a high level of canopy-intercepted radiation, a low diurnal temperature range and low levels of evapotranspiration in the understorey; and (iv) the economically mature stage (25-30 years) may feature open pockets, with patches exposed to more variable radiation and evapotranspiration (Walter and Himmler 1996). Wind-throw, pruned branches and waste thinned trees adds woody residue habitat to the local forest floor architecture, providing microhabitats for a variety of microbial, fungal, micro and macroarthropod species (Allen *et al.* 1995, Humphrey *et al.* 2000).

It is noted that these categories are unlikely to fully address all the possible stages of a stand's development. For example, "felling and replanting" is an important part of the forestry cycle and can be expected to have substantial impacts on the forest floor biota and soil physics.

The species diversity/habitat heterogeneity theory predicts a positive relationship between species diversity and habitat complexity. An underlying assumption is that habitat complexity increases with time. It was predicted that species diversity would be linearly related to stand age, such that older stands would have greater diversity than younger stands". Do forest stands become more or less spatially homogenous with time and if so, what is the effect on invertebrate diversity? An answer to these questions is of both theoretical interest and practical value. The outcome may not only provide ultimate explanations for the invertebrate community assemblage in the forest, but also validate reasons for specific management activities that could increase local invertebrate diversity

There are also repercussions for local and regional diversity and forest productivity (Butterfield 1997, Hanowski *et al.* 1997). If more complex (heterogeneous) habitats support a more diverse species assemblage, it might be expected that ecosystem function is enhanced in such sites

(Lawton 1994) (Jones and Lawton 1995, Wardle and Lavelle 1997). The positive linkages forged between ecosystem function and productivity (Kangas and Kussipalo 1993, Lavelle *et al.* 1997) should then be sufficient to encourage resource managers to employ sustainable practice to optimize biodiversity and the adherent ecological services provided by components of that diversity (Franklin *et al.* 1989, Schulze and Mooney 1992, Swift and Anderson 1993, Naëem *et al.* 1994, Mineau and McLaughlin 1996, Heneghan and Bolger 1998). Support for this theory could strengthen the argument for general laws in ecology.

In this chapter, the diversity of the soil and litter arthropod assemblage from four *P. radiata* forests was used as a metric to evaluate the relationship between arthropod species diversity and the stage of development of a stand. I found the relationship between arthropod species diversity and stand development was best described logarithmically ($r^2 = 0.323$). Diversity in the planted forests increased most rapidly during the first 10 years of development. Detrended Canonical Analysis identified a distinct split in species affiliations between the establishment age class and subsequent stages of stand development. Indicator Species Analysis identified suites of species, which were both significant and typical of the stage of stand development. These outcomes are discussed in relation to support for the habitat heterogeneity theory and the management for biodiversity at the local scale.

B. MATERIALS AND METHODS

1. Study sites

Between early December 1999 and late January 2000, four independent forests within the Dunsdandel-Hororata district were surveyed using both pitfall traps and litter extraction techniques. Each stand was selected as being representative of a progressive stage of development within this locality, with canopy features which were fairly well correlated with the arbitrary designations of Lewis (1993). The maximum distance between stands was approximately 8 km and the minimum distance was approximately 4 km. The age classes at the time of sampling (1999) were:

(i) Establishment: "Mitchells" is a 1-year-old stand replanted in 1999. Between tree lines, the mineral soil was substantially exposed with some small gorse and grass seedlings present. The stocking rate was 1250 stems/ha.

(ii) Pre-canopy: "Burgess" is an 11-year-old stand (replanted in 1989) following a fire. Trees were grass released with herbicide in 1989 and 1990. The stand was pruned in 1997 and waste thinned in 1999 and an understorey of grass and gorse were present. In 1998 the stocking rate was 1138 stems/ha.

(iii) Post-canopy: "Bennetts" is an 18-year-old stand (replanted in 1981) which was pruned and thinned in 1990. A scattered understorey of wattle was present. In 1997 the stocking rate was 686 stems/ha.

(iv) Mature: "Wattle" is a 24-year-old stand (replanted in 1975) which was pruned and waste thinned in 1983. A scattered understorey of wattle was present. In 1996 the stocking rate was 636 stems/ha.

The study sites were not replicated. The experimental design was designed to provide an accurate picture of the arthropod assemblage from each age class category. The outcomes were therefore not extrapolated beyond the study sites and any inferences are made with acknowledgement of pseudoreplication. Arthropod samples were taken from replicated traps within each stand. A total of 12 pitfalls were placed in each stand, and left open continuously for 6 weeks. Placement of pitfalls was along mid row transects throughout each stand, such that no trap was closer than 20m from its' nearest neighbour. Similarly, litter samples were taken from mid row transects at 20m intervals and previous sampling sites marked such that no sample was taken within 10m of a pitfall trap or 10m of a previously sampled area.

The catch from each pitfall was cleared weekly and bulked to represent the total catch for that site for that week. The weekly clearance was to ensure trap preservatives remained effective. At each stand, 6 litter samples were taken at 7-day intervals using a 10 cm x 10 cm grid. All material within the grid and mineral soil to a depth of 5 cm was included in each sample. The weekly litter samples were independently bulked for each stand and processed.

A full description of pitfall construction, litter extraction and invertebrate identification techniques was previously given in Chapter Three.

2. Analysis

A taxonomic inventory was collated. The mean abundance (±SD) of arthropods within each taxon and the total number of individuals within an Order trapped in (i) pitfalls or (ii) extracted from litter was calculated. The Shannon-Wiener Diversity Index (H') and Sorenson's Similarity Index were used to assess species diversity and the degree of similarity between the four forest sites. The formulae and interpretation of these measures is provided in detail in Chapter Three.

The data were examined by regression to establish and estimate the dependence of species diversity (H') on stand age. A graph of the residual versus actual values was visually assessed to establish the evenness of the residual distribution throughout the data range. A curve was fitted to

the actual values to identify the strength of the relationship between the variables. Exploratory data analysis (DCA ordination, PCOrd) was used to identify key groupings of sites, using scatterplots, according to the invertebrate species trapped and the method of trapping. Two Way Indicator Species Analysis (TWINSPAN) (Hill and Gauch 1980) was used to group and classify samples. Indicator Species Analysis (Dufrene and Legendere 1997) was then used to provide a list of statistically significant species and their Indicator Value. Detailed information on TWINSPAN and Indicator Species Analysis is given in Chapter 3.

C. RESULTS

1. The effectiveness of different trapping methods

The total number of individuals and the composition of the arthropod assemblage trapped by pitfalls varied between the different age classes. In the establishment age class, 2672 individuals were trapped. Phalangids were the dominant order, accounting for 80% of all individuals taken. In the pre-canopy age class, 244 individuals were trapped; the numerically dominant orders were the Arachnida (33%) and Collembola (31%). In the post-canopy age class, 1509 individuals were taken; the numerically dominant orders were the Collembola (35%) and the Orthoptera (24%). In the mature age class, 1672 individuals were extracted; the sample was dominated numerically by the Diptera (42%).

The total number of individuals and the composition of the invertebrate assemblage extracted from litter samples varied between the different aged stands. In the establishment age class, 98 individuals were extracted, of which 60% were from the order Coleoptera. In the pre-canopy age class, 244 individuals were extracted, with 61% of the catch being collembolans. In the post-canopy age class, of the 1797 individuals extracted, 63% were collembolans. In the mature age class, 4046 individuals were extracted from the litter, of which 87% were collembolans.

2. Previously unaccounted-for species

Pitfall traps accounted for eight additional species not previously reported in this thesis. Litter sampling did not account for any additional taxa. The new taxa were all present in very low abundance (3 or fewer individuals trapped) and they included the arachnids *Rinawa canturaria* (Hahnidae), *Laetesia* RTU1 (Linyphiidae), *Hemicloea rogenhoferi* (Gnaphosidae) and the coleopterans *Bethelium signiferum* (Cerambycidae), *Lithostignus* sp. (Corticariidae), *Sitona discoideus* (Curculionidae) and *Mimopeus elongatus* (Tenebrionidae).

3. Shannon-Wiener Diversity (H')

Shannon-Wiener diversity (H') for invertebrates sampled by (i) pitfalls, (ii) litter and (iii) pitfalls and litter, at each of the four forest stands is summarized in Table 4.1. The combination of data from pitfalls and litter extraction gave the most clear and significant demarcation in the differences in diversity (H') between the establishment age class, compared with the pre-canopy, post-canopy and mature age classes (ANOVA, $F = 11.31$, $P < 0.001$). The lowest level of diversity (H') was found in the establishment age class and the highest level of diversity (H') was found in the mature age class. Litter sampling accounted for a greater range in diversity (H') across age classes than did pitfall sampling.

Table 4.1 Mean Shannon-Wiener Diversity (H') of invertebrates in pitfall or litter samples at each of the four sampled age classes: establishment (1-year), pre-canopy (11-year), post-canopy (19-year) and mature (24-year). Values are the mean (±SD) diversity (H') for replicate samples using either pitfall or litter data and pitfall and litter data combined for each site. Values within a single column without a common letter differ at the 5% level as per Tukey's Multiple Comparison test of means following ANOVA.

Stand age class (years)	H' (±SD) Pitfalls (N = 12)	H' (±SD) Litter (N=6)	H' (±SD) Pitfalls and Litter (N= 18)
1	1.11 (±0.54)[a]	0.50 (±0.66)[a]	0.91 (±0.64)[a]
11	1.77 (±0.33)[b]	1.15 (±0.73)	1.57 (±0.56)[b]
19	1.51 (±0.07)	2.01 (±0.61)[b]	1.68 (±0.65)[b]
24	1.92 (±0.22)[b]	2.08 (±0..38)[b]	1.95 (±0.28)[b]

4. A model linking species diversity (H') with the stage of development of a stand

The strength of the association between Shannon-Wiener diversity (H') (pitfall and litter samples combined) and stage of stand development was more variable in the establishment, pre-canopy and post-canopy age classes than in the mature age class stand (Figure 4.1). The logarithmic curve fitted to the H' values for the four age classes indicated a positive association between invertebrate diversity and stage of stand development (coefficient of determination = 0.3091, $P < 0.001$, $F = 31.31$, $df = 70$) (Figure 4.1). I made a visual examination of the residuals from the regression to identify potential bias. Outliers < 2 or > -2 were not rejected (Fry 1994) as they were interpreted as being the extreme values of an invertebrate community data set which had previously been observed (see Chapter Three) to be quite variable within replicate plots. In some cases, nil invertebrates were trapped. Several trial curves were fitted to the data, with the line of best fit which was selected (a logarithmic curve) offering both the most biologically reasonable pattern and the highest coefficient of determination (coefficient of determination = 0.323) (Figure 4.2).

The logarithmic model suggests Shannon diversity (H') is lowest at the beginning of a rotation and highest after 24 years when the stand is reaching maturity. The most rapid increase in

diversity occurs in the `first 10 years of establishment. In subsequent years, there is a gradual increase in diversity (H') as the forest matures.

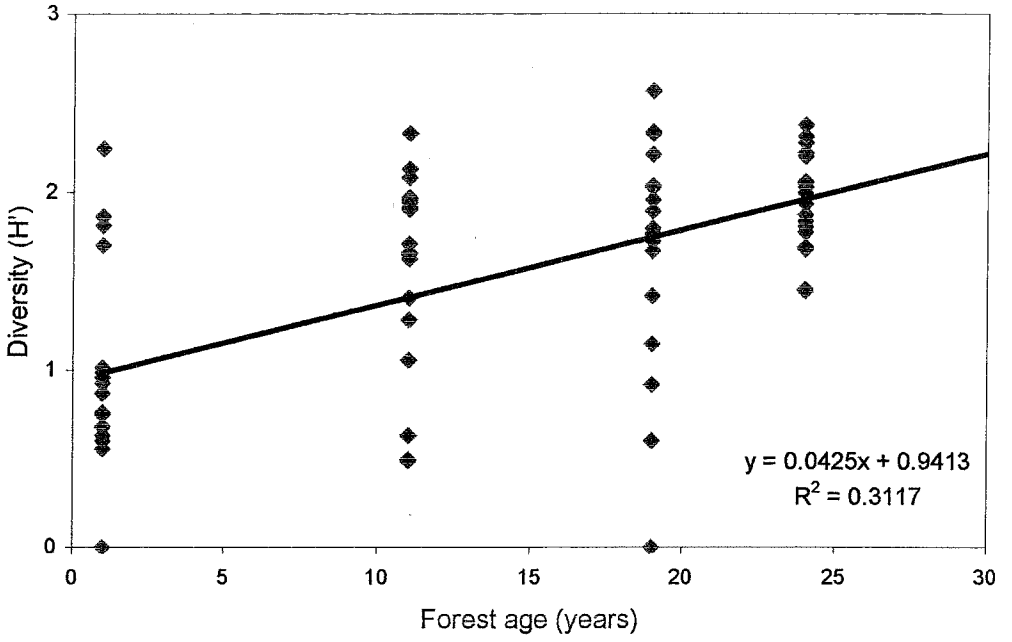

Figure 4.1 Simple plot with linear regression line fitted to the Shannon-Wiener Diversity (H') data for combined pitfall and litter catches from each of the four sampled *P. radiata* age classes representing a gradient from 1 to 24-years.

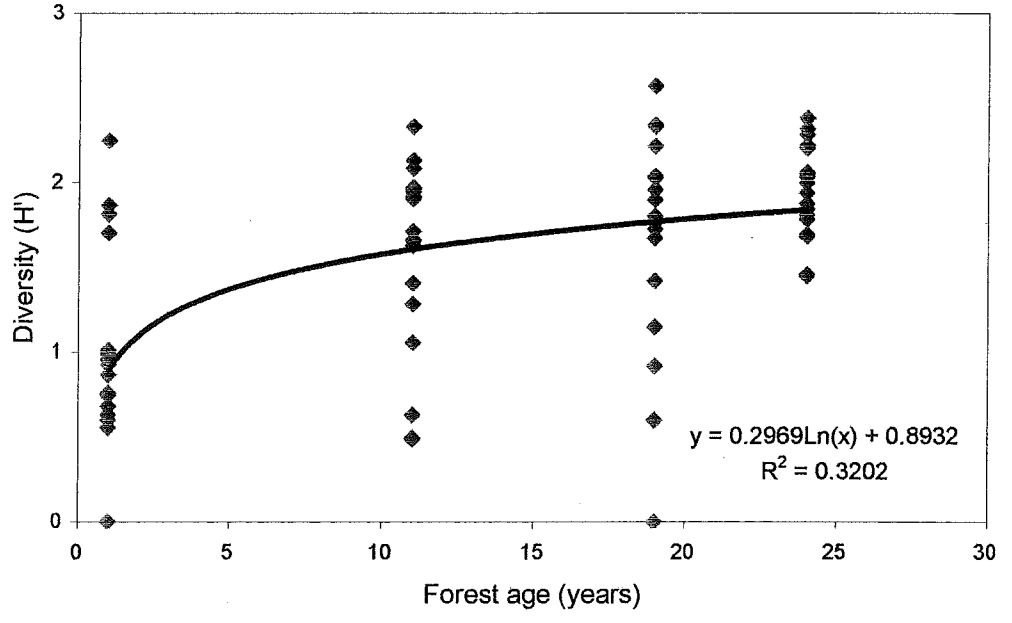

Figure 4.2 Simple plot with logarithmic regression line fitted to the Shannon-Wiener diversity (H') data for combined pitfall and litter samples from each of the four sampled *P. radiata* age classes representing a gradient from 1 to 24-years.

5. The similarity of the species assemblage across age classes

Sorenson's Similarity Index indicated a higher than average level of similarity (> 0.5 Sim.) in beta diversity between each possible pair for each of the four sampled age classes (Table 4.2). The post-canopy and mature age classes had the greatest amount of species in common (Sim. 0.81), indicating a low turnover of species between these stand age classes. The establishment and mature age class had the least amount of species in common (Sim. 0.60), indicating a high turnover of species occurred between the initial stage of stand development and stand maturity.

Table 4.2 Sorenson's Similarity Values for the four sampled age classes: establishment (1-year), pre-canopy (11-year), post-canopy (19-year) and mature (26-year). Values derived from presence-absence matrix for both pitfall and litter-trapped invertebrates at each site.

Sorenson's Similarity Values			
Age class (years)	**24**	**19**	**11**
19	0.81		
11	0.76	0.74	
1	0.60	0.63	0.64

6. DCA Ordination of invertebrate assemblages

The DCA ordination split the pitfall catch data into two main groups (Figure 4.3). Group 1 incorporated the pre-canopy, post-canopy and mature age classes. Group 2 was composed solely of replicates from the establishment age class. Axis 1 represents the different species composition from pitfall-only trapping within each age class along an age gradient. The age gradient is reflected from left to right along Axis 1. There was a high level of overlap in species' assemblages in the three older age classes. There was a clear demarcation in species association between the three older age classes and that of the establishment age class.

The DCA ordination split the litter catch data into two similar main groups (Figure 4.4). Group 1 consisted of the pre-canopy, post-canopy and mature age classes. There was a high level of overlap in the age gradient between the pre-canopy, post-canopy and mature age classes. The establishment age class (MIT) was stretched widely along Axis 1 with two replicates firmly associated with the pre-canopy post-canopy and mature age classes. There was a clear separation in species association between these three age classes and the establishment age class.

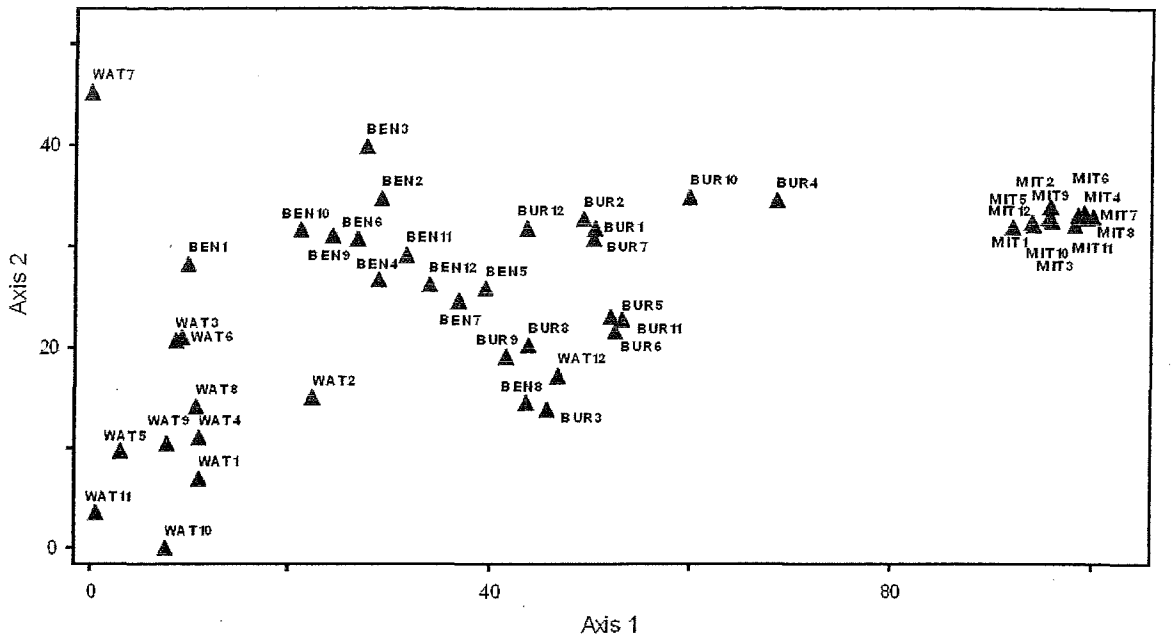

Figure 4.3 DCA ordination of the four forest sites grouped by pitfall data. TWINSPAN separated the four stands into TWINSPAN separated the four stands into two distinct age class groups. These were Group 1 (11, 18 and 26 years) and Group 2 (1 year). MIT = Mitchells, BUR = Burgess, BEN = Bennetts, WAT = Wattle. Numerical values after abbreviations represent replicate samples.

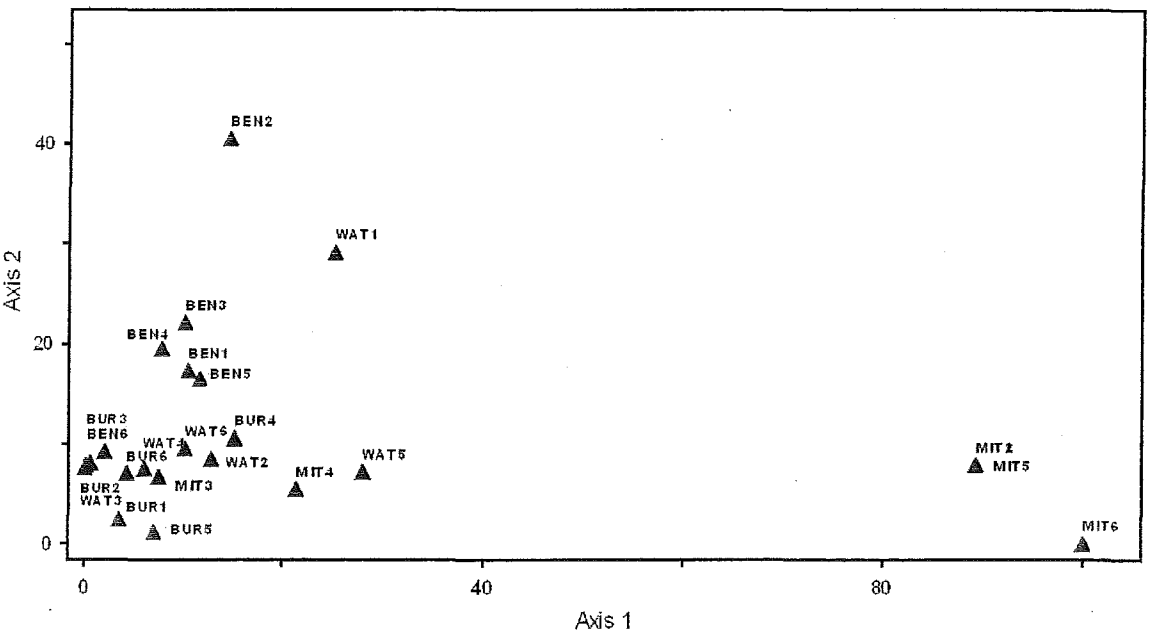

Figure 4.4 DCA ordination of the four forest sites grouped by litter data. TWINSPAN separated the four stands into two overlapping age class groups. These were Group 1 (11, 18 and 26 years) and Group 2 (1 year). MIT = Mitchells, BUR = Burgess, BEN = Bennetts, WAT = Wattle. Numerical values after abbreviations represent replicate samples.

7. Indicator Species Analysis

The Indicator Species Analysis (ISA) for the ten most typical taxa trapped by pitfall only in the TWINSPAN-derived groups is summarized in Table 4.3. The species most typical of Group 1 (pre-canopy, post-canopy and mature age classes) with an IV > 50% included the web spinning predator *Steatoda capensis* (IV 83.3%), the scavenger *P. scaber* (IV 77%), the small huntsman *Nuncia* sp. (IV 75%), the endemic adult craneflies *L. tapleyi* and *L. zeylandiae* (IV 61.1%), staphylinid beetles from the sub family Oytellinae (IV 53.6%) and small linyphiid spiders from the sub family Mynogelninae (51.5%).

Group 2 taxa with an IV > 50% included the long-legged harvestman *Phalangium opilio* (IV 100%), *Agrypnus variabilis* (IV 75%), collembolans (IV 73%), the introduced money spider *Diplocephalus cristatus* (IV 74.5%) and the indigenous wolf spider *Lycosa hilaris* (IV 71.4%).

Table 4.3 The ten most typical taxa from pitfall traps ranked on their Indicator Value suggested to be indicative of either Group 1 (11, 18 and 26 year age class categories) or Group 2 (1 year age class category).

Group 1				
Taxon	**IV**	**Mean**	**SD**	**P value**
Steatoda capensis	83.3	17.9	6.09	0.001
Porcellio scaber	77	42.3	8.95	0.002
Nuncia sp.	75	36.1	6.75	0.002
Leptotarsus zeylandiae	61.1	32.3	7.52	0.002
Leptotarsus tapleyi	61.1	31.1	7.18	0.003
Staphylinidae: Oxytelinae RTU1	53.6	30.7	7.14	0.012
Linyphiidae: Mynogleninae RTU1	51.5	35.5	7.33	0.036
Aparua kaituna	44.4	23.9	6.26	0.008
Forficula auricularia	44.4	24.6	6.85	0.018
Pleioplectron simplex	33.3	19.6	6.32	0.047
Group 2				
Taxon	**IV**	**Mean**	**SD**	**P value**
Phalangium opilio	100	20.8	6.46	0.001
Agrypnus variabilis	75	16.1	5.7	0.001
Collembola	73	56.9	5.4	0.005
Diplocephalus cristatus	74.5	32.4	6.67	0.001
Lycosa hilaris	71.4	44.9	7.57	0.005
Coccinella undecimpunctata	41.7	11.2	5.08	0.001
Curculionidae RTU1	41.1	13	5.13	0.002
Pristoderus antarcticus	41.7	11.5	4.82	0.001
Aphodius tasmaniae	37.5	13	5.19	0.004
Microtenonyx subitaneus	25	7.8	3.33	0.008

The Indicator Species Analysis (ISA) for the ten most typical taxa trapped by litter only in the TWINSPAN-derived groups is summarized in Table 4.5. Only two taxa, the Collembola (IV 95%) and spiders from the sub family Mynogleninae (IV 85%) were significant indicators of

Group 1; only the non predatory coleopteran larvae (IV 73.5%) were significant indicators of Group 2.

Table 4.4 The ten most typical taxa from litter ranked on their Indicator Value suggested to be indicative of either Group 1 (11, 18 and 26 year age class categories) or Group 2 (1 year age class category). *Non-predatory species.

Group 1				
Taxon	**IV**	**Mean**	**SD**	**P value**
Collembola	95	63.7	14.65	0.003
Linyphiidae: Mynogleninae RTU1	85	50.5	11.63	0.003
Gamisiidae	70	46.1	12.69	0.055
Sciaridae (larvae)	65	46.4	13.09	0.094
Neotrichozetes RTU1	60	40.8	12.88	0.099
Staphylinidae: Oxytelinae RTU1	60	39.7	12.35	0.098
Staphylinidae: Aleocharinae RTU1	60	39.7	12.05	0.086
Tipulidae (larvae)	55	37.2	12.06	0.143
Staphylinidae (larvae)	55	38.5	12.58	0.146
Cylindrioulus britanicus	50	37.4	12.76	0.225
Group 2				
Taxon	**IV**	**Mean**	**SD**	**P value**
Coleoptera (larvae)*	75.3	51.2	11.83	0.042
Steatoda capensis	20.8	13.8	7.41	0.287

D. DISCUSSION

New species contributing to site biodiversity

Eight additional species were trapped which had not previously been reported in this thesis, These new species were represented by either singletons or fewer than 3 individuals. New arachnids included the indigenous, predatory spider *Rinawa cantuaria* Forster 1970 (Hahnidae) and the tiny sheet web spider *Laetesia* RTU1. *R. cantuaria* has previously been recorded in a survey of arthropods of Banks Peninsula (Johns 1986) and may be restricted to the Canterbury region (Forster 1970). The flat bark spider *Hemicloea rogenhoferi*, a widespread species introduced from Australia that tends to prefer bark and log shelters was also trapped (Johns 1986, Macfarlane *et al.* 1999). New coleopterans included the forage pest weevil *Sitona discoideus,* currently the subject of a biological control programme in the Canterbury Plains (Kean and Barlow 2000) and a singleton *Lithostignus* RTU1, which is most probably mycetophagous. The phytophagous beetle *Bethelium signiferum* and the generalist scavenger tenbrionid *Mimopeus elongates* were also present.

Biodiversity challenged by habitat disturbance

This survey confirmed a high proportion of adventive and opportunistic species with general distributions, some of which were indigenous and most of which were recognized to be tolerant

of habitats modified by humans (Johns *et al.* 1980, Klimaszewski and Watt 1997). Many of these species could be expected to rapidly recolonize sites following normal forestry operations, although some, with more selective habitat requirements may be challenged. Examples include cryptic species such as the larval craneflies, dependent on the fungal and microbial resource which is present in accumulated litter. Because many species were represented by only a few individuals, it is impossible to ascertain whether these species were weakly represented in the sample because of rarity or because they were not easily trapped by the methods used. Good examples of this dilemma are *Rinawa cantuaria* and *Laetesia* spp. Their low abundance may also suggest habitat intolerance or even reflect normal population fluctuations.

Limitations of a short, season-specific sample

Although the "snapshot in time" sampling effort was of a short duration and highly season-specific, the timing of sampling (late summer) probably accounted for most of the ground active species present. However, the idiosyncratic behaviour of some species, such as the adult craneflies *Leptotarsus tapleyi* and *L. zeylandiae* highlights the need to understand species life histories to adequately account for their contribution to biodiversity. Had sampling been undertaken outside of the short temporal window (approx. 14 days) in which they emerge, these species would have been missed. Adult craneflies are very readily trapped by pitfalls but better estimates of their abundance would be expected using malaise traps. Although the use of a short and intensive sampling effort has previously been shown to be effective for malaise-trapped beetles in pine forests (Hutcheson and Jones 1999) especially where flight behavior tends to be most active during mid summer, short sample periods may not provide the most inclusive database where a more generalist approach to biodiversity is taken.

Taxon sampling curves

A taxon sampling curve was not used in the short survey presented in this Chapter. The validity of diversity comparisons between sites has been questioned in the absence of a taxon sampling curve (Gotelli and Colwell 2001). However, I am confident that the sample is a fair representation of the species present, because there is generally a good concordance in the species representation between this brief survey and the two surveys (one of which spanned four successive seasons) presented in Chapter Three. For this reason, Shannon-Wiener diversity (H') Index presented is believed to be valid as it reflects fair species representation for these sites.

A logarithmic model of diversity (H')

The logarithmic curve describing the incremental relationship between species diversity and the stage of stand development is biologically realistic, given the acknowledged changes in stand architecture which can be strong determinants of invertebrate presence and activity (McColl 1974a, Lewis and Ferguson 1993). Thus, the logarithmic model suggests there are two-stages of development in the invertebrate assemblage associated with the planted forests in this locality. These two stages are an initially rapid increment in diversity in the first (approx.) 10 years which gradually tails off to a plateau in the latter years. It is interesting to speculate on some degree of correlation between the commencement of this plateau and management activities such as pruning and waste thinning.

The choice of the logarithmic model is further underpinned by the significant dissimilarity in beta diversity (i.e. species turnover between sites) for combined pitfall and litter samples from the establishment age class and all other age classes. Furthermore, there is a stepwise increment in H' from the youngest through to the oldest sampled stand.

It is important to note that this model of invertebrate diversity has limited similarities with observations of plant species richness after forests are clearcut (Gove *et al.* 1992, Lugo 1992). The only comparable New Zealand study (at Kinleith forest in the North Island) observed plant species richness to be highest immediately after harvesting, declining at the 13 year stage and then increasing towards the end of the rotation (Allen *et al.* 1995).

The pattern of developing diversity

The low starting point value of diversity (H') in the establishment age class follows the harvest of the previous rotation and the subsequent post-harvest site preparation. It is probably due to the removal of biomass, simplification of habitat structure and exposure of sites to incoming radiation and precipitation as well as the drying and cooling effects of wind (Gadgil and Gadgil 1978, Hawke and Wedderburn 1994, Humphrey *et al.* 1999, Bird *et al.* 2000, Werner and Raffa 2000).

During the establishment period, opportunities exist for site recolonization by an invertebrate assemblage drawn from nearby stands, from populations in windrows left from earlier rotations, from residual populations in the soil, and strong flying species (Bonham *et al.* 2002). I found the invertebrate assemblage in the establishment age class stand to be best characterized by ground active and highly mobile, opportunistic predators, for example linyphiid spiders, the harvestman *Phalangium opilio* and *Lycosa hilaris*. Such species feature a variety of traits, both behavioural

and physical, which make them adept colonizers. For example, the female *L. hilaris* transports her young and may progressively distribute offspring across the habitat. The general absence or low abundance of cryptic and mycetophagous species is probably due to the large areas of exposed mineral soil, lack of damp rotting wood or accumulated litter, which both limit the resources available and increase the risk of exposure and desiccation.

It is reasonable to expect that the microhabitat of an establishment age class stand will be subject to more extreme diurnal and seasonal variation than a mature stand (see Chapter 3, Figure 3.1). An exposed, seasonally-dry habitat could favour some ground-active predatory carabids (Jukes *et al.* 2001). These coleopterans have previously been noted as early colonizers of clear felled British conifer forests (Butterfield 1997). Their presence reflects prey availability. Most of the carabids trapped in the reported survey were ground-active predators (similar findings are reported in Chapter 3, Tables 3.3 and 3.4). Within-site microhabitat variation (contributed to, for example, by tree-row shading or understorey plant growth) may variously favour mycetophagous and phytophagous coleopterans (Mason *et al.* 1988, Walter and Himmler 1996, Vohland and Schroth 1999, Tanabe *et al.* 2001). For example, it is likely that the under storey grasses and woody weeds provided habitat for the phytophagous curculionid *Steriphus diversipes,* which was abundant in the establishment phase.

The rapid acceleration in diversity during the establishment period observed in the mid Canterbury forests, has also been noted in Texan pine plantations (Bird *et al.* 2000). In that study, a very rapid return to normal levels of invertebrate abundance and species diversity occurred within 12 months of mechanical harvesting of stands. This was attributed to the recovery of the undergrowth vegetation, which provided protection from adverse abiotic conditions, more food resources for phytophagous and detrivorous species and an increase in microhabitat stability.

The distinctive turnover in species composition shown in the mid Canterbury study, occurs at about the time most stands in this locality are pruned and waste thinned (i.e. 8-10 years after planting). The woody waste and needles residues add to the structural complexity of the forest floor, supply nutrients for the decomposer species and shelter for others (Marra and Edmonds 1998). For example, the scavenging dermapteran *Forficula auricularia,* which was absent in the establishment age class, progressively increased in abundance after this stage of stand development. Pruning improves access and stands may be treated with herbicides to suppress competitive weed species, thereby reducing the complexity of the understorey architecture.

The progressive closure of the canopy imposes light limitations on under storey growth and also intercepts increasingly greater amounts of the available precipitation (Bonham *et al.* 2002). Canopy closure is understood to contribute to a more stable, homogenous microclimate, which is generally cooler and lacks the variation in the diurnal temperature range evident in younger stands. Substantial accumulations of needle litter occur in the late pre-canopy and post-canopy stages, facilitating more complex trophic interactions between litter and ground-dwelling species. For example, fungal communities develop extensive mycelial networks in the needle litter (Lamb 1976, Butterfield 1999) providing food resources for mycetophagous species. I found populations of collembolans to be most abundant in litter samples in the post-canopy and mature age classes. These species are preyed upon by predatory staphylinid larvae (Poole 1961, Hopkin 1997) which were abundant.

The lateral stratification of decomposing litter into horizons was likely to have influenced the diversity and abundance of mites (Poole 1961, Anderson 1975, Luxton 1981, Hansen and Coleman 1998) because successive layers reflect different stages of decomposition and nutrient quality (Styles 1967, Forrest 1969, Anderson and Ineson 1984). I found the mean abundance of the Camisiidae, Chamobatidae, Neotrichozetidae, Parasitidae, Rhodacaridae and prostigmatid mites increased with the stage of development of a stand. A stable microclimate may partially explain the increased occurrence of the Camisiidae and Neotrichozetidae families, as both are sensitive to moisture stress and are generally found in association with cool habitats (Luxton 1981).

Macro- and microecology

Interpretation of the observed trends in species diversity (H') in relation to spatial heterogeneity is entirely dependent on the observational scale and grain of the investigation (Heneghan and Bolger 1998). Substantial differences in diversity become evident when the scale shifts from alpha (within sites) to beta (between sites) or gamma (between regions). It is interesting to note that the observed pattern of beta diversity that has emerged from this study has enabled generalizations, which are often difficult to find in community-level ecology (Lawton 1999). However, those generalizations are only useful if they provide immediate and practical solutions to specific problems. In terms of this study, the problem may be finding ways to maintain or enhance local biodiversity. If the overall pattern of change is predictable, then management options can be targeted towards this endpoint.

The complexity of the space immediately adjacent to the forest floor may be high in the early years of stand development because of the structure and composition of the under storey vegetation. However, with time, the components of that space change as the stand progresses to economic maturity, when the under storey vegetation generally simplifies, litter complexity increases and the microclimate stabilizes under a protective canopy In the case of these mid Canterbury stands, the under storey vegetation (woody weeds and grasses) generally present in younger stands is progressively supplanted by debris from waste thinnings, pruned material, accumulated needle litter and windthrow. If the habitat heterogeneity theory is assumed to be true, then the mature forest floor must also be assumed to boast a greater spatial heterogeneity than that present in a younger forest.

However, in order to apply this relationship to management, it may be beneficial to consider the logarithmic shift in diversity (H') with time as being a resource-based response, rather than a response to spatial complexity. Resource-based responses have been shown for mite communities where invertebrate species diversity increased in relation to the amount and proximity of woody debris on the forest floor (Marra and Edmonds 1998). Similarly, litterbag studies using mixed and simple litters identified the mixed litters to have the greatest variety of microhabitats (as defined by substrate type and fungal growth form) and to support a greater species richness of oribatid mites (Hansen and Coleman 1998).

Future studies examining the habitat heterogeneity theory in the context of invertebrate diversity in the planted forest should quantify the meaningful structural components of the invertebrate habitat (i.e. the components which resources). For this to be effective, an understanding of specific life histories and species biologies would be necessary. To minimize the complexity of such an investigation, the indicator species approach using a suite of typical species should prove invaluable. In this way, it may be possible to identify general laws in ecology on a local scale which could facilitate local management decision-making processes.

Monitoring the effects of management on species diversity

The use of invertebrate species, which exhibit radical fluctuations in their spatial distribution, may prove risky bioindicators of effect at scales greater than micro site. For example, synchronized decomposition of uniform substrates could promote a "boom or bust" economy in microbial resources, which in turn may affect the abundance and distribution of fungivorous species, such as the collembola (Hansen and Coleman 1998). For this reason, the collembola were excluded from the recommended list, despite being typical of the later stages of stand

development. Other studies have utilized collembolans to effectively measure ecological effects in forest habitats (Poole 1961, Butterfield 1999). However, their size and some difficulties associated with taxonomy does constraint their suitability. Another exclusion was the weevil *Agrypnus variabilis.* This is because although it was highly typical of the establishment age class, it was present in very low abundance.

Ecologically meaningful indicators for future assessments

The Indicator Species list provided by the ISA analysis fulfilled the basic requirements of surrogate measures of biodiversity, as all species were abundant, ecologically meaningful, amenable to measurement by non-specialists and believed to be applicable at the local scale (Ferris-Khan *et al.* 1998). For this locality and these forest habitats, the recommended methodology for trapping is by pitfalls and the species suggested to be the most effective indicators are:

(i) Establishment age class (approx. 1-10 years): *Phalangium opilio, Diplocephalus cristatus* and *Lycosa hilaris*

(ii) Pre canopy, post canopy and mature age classes (>10 years): *Steatoda capensis, Porcellio scaber, Nuncia* spp., *Leptotarsus zeylandiae* and *Leptotarsus tapleyi* and staphylinid beetles

This recommended list includes both introduced and indigenous species. The staphylinid beetles have previously been proposed as suitable bioindicators in European studies where the taxonomy is better understood (Bohac 1999). There are taxonomic limitations as far as the New Zealand species are concerned (Klimaszewski and Watt 1997). Similarly, many of the New Zealand tipulids have yet to be formally described. However, the two species suggested here are readily identifiable (P.M. Johns unpublished data) by visible structural differences in the antennae and the brachyptery of the female *L. zeylandiae.*

Conclusions

The spatial structure of the forest floor structure is an age-related characteristic, which varies in relation to the type, quality and variety of components providing a resource for forest floor invertebrates. Support for the habitat heterogeneity theory was found, in which a logarithmic model generated from the survey of selected stands indicated an increase in the H' of invertebrates which increased rapidly during the initial (approx.) ten years, gradually approaching an asymptote as economic maturity occurred.

Development of this model was constrained by the decision to either conduct a robust and detailed experiment across a limited number of sites, or to use a greater number of less rigorously designed sampling events across a large number of sites. The model is limited because only four sites representing a gradient of age classes were used and the experimental design provided detailed information on a much localized scale. Therefore, its applicability beyond mid Canterbury remains questionable. However, the model effectively defines a local scale pattern which is not often easily found in community level ecology.

For the mid Canterbury forests to be managed for their contribution to local invertebrate diversity, three recommendations are that; (i) forest blocks be managed such that maturing and mature stands are interspersed with new stands to provide founder populations following harvesting and site preparation; (ii) that intensive management activities within stands up to approx. 10 years of age be sensitive to this period of active colonization and growth of invertebrate populations. This is because subsequent tree growth may be dependent on nutrient cycling mediated by some of these soil and litter-dwelling species and; (iii) that intensive management activities within any stands be cognizant of the potential for alterations to the normal structural components of the forest floor to influence the abundance and representation of the current species assemblage.

CHAPTER FIVE

LINKING SPECIES ABUNDANCE TO ECOSYSTEM FUNCTION: TIPULID LARVAL ABUNDANCE IN RESPONSE TO BIOSOLIDS APPLICATION AND THEIR CAPACITY TO ENHANCE SOIL AERATION

A. INTRODUCTION

The larvae of the cranefly family, Tipulidae (Diptera: Nematocera) utilize a wide variety of both terrestrial and aquatic habitats (Skerman 1953, Freeman 1967, McCracken *et al.* 1995, Frouz 1999). Commonly known as "leatherjackets", the terrestrial species are found in abundance in habitats characterized by damp soils, moss and rotting woody debris (Coulson 1959). Their trophic status varies according to species, and includes specialist bryophytovores, generalist detrivores fungivores and herbivores (Freeman 1967, Hoevemeyer 1996, Frouz 1999, Smith *et al.* 2001).

The perceived ecological value of the larvae varies greatly, from pest status to a wildlife resource (Schiegg 2001). For example, in Britain, the phytosaprophagous *Tipula oleracea* Linnaeus. (Tipulidae: Tipulinae) and *T. paludosa* Meigen (Tipulidae: Tipulinae) are both serious crop and pasture pests (Meats 1972, Service 1973, Vlug and Harrewijn 1994, Frouz 1999), whereas two common grassland, woodland and forest species *Limonia nubeculosa* Meigen (Tipulidae: Limoniinae) and *Tipula scripta* Meigen (Tipulidae: Tipulinae) are important prey for many birds (Service 1973, McCracken *et al.* 1995, Wilson *et al.* 1999). In laboratory and field experiments, tipulid larvae have been shown to contribute to decomposition of litter fall, increase the reducibility of litter, its ash content and the mobility of organic matter; they also facilitate the penetration of microorganisms into deeper layers (Perel *et al.* 1971, Smith 1989). My own observations include a negative phototaxic response, and the development of short horizontal tunnels in weakly compacted soils. There is no known documentation referring to their behaviour in mineral soils as occurs in the Canterbury plain.s

In forest and woodland ecosystems in particular, the distribution and abundance of craneflies has been correlated with the presence of a well-developed litter layer (Paquin and Coderre 1997, Frouz 1999, Young and Koenig 2000), where the moderated soil microclimate supports a complex community of microbial and fungal decomposers (Meir *et al.* 1995, Vesterdal *et al.* 1995, Rafferty *et al.* 1997). Under such conditions, both cranefly larvae and earthworms may

represent a substantial proportion of the surface and topsoil-dwelling invertebrate species (Lukasiewicz 1996). However, unlike earthworms, cranefly larvae display a high level of tolerance to soil acidification (Freeman 1967, Wallwork 1970, Yeates and Saggar 1998, Yeates *et al.* 2000). Furthermore, the larvae are also found where other soil characteristics (such as high bulk density or low organic matter) are limiting to earthworms. A model generated to explain the distribution of *Tipula* spp. larvae in pastures in Scotland indicated none of the standard soil characteristics, such as pH, bulk density or percentage loss on ignition, had any effect on leatherjacket numbers (McCracken *et al.* 1995).

New Zealand's endemic cranefly genus *Leptotarsus* spp. (Tipulidae: Tipulinae), are widely encountered, as adults, across the east coast of the South Island. The genus has been trapped in a variety of habitats, including tussock grasslands, podocarp and broadleaf forests, exotic planted forests, seepages and swamp grasses (P.M. Johns, unpublished data). Both cranefly larvae and the indigenous Megascolecidae (earthworm) fauna of the east coast are typically found in association with a plentiful supply of organic debris (Lee 1959, Lukasiewicz 1996) and probably co-existed under native vegetation in the past. Although the latter have all but retracted to isolated remnants of indigenous vegetation (Lee 1959) cranefly larvae retain a broad distribution.

The general absence of a well-defined or characteristic native earthworm fauna in New Zealand has been attributed to deforestation, pasture improvement, habitat modification and the introduction of European earthworms (Lee 1959, Yeates 1991). A similar situation for surface dwelling earthworms has been noted in France (Lee 1985). In fact, agricultural soils worldwide contain very few indigenous earthworms, as they have been colonized by a suite of about a dozen peregrine species (Doube and Schmidt 1997).

Since the time of early settlement, the Canterbury plains have undergone extensive habitat modification. The extensive, arable landscape of mid Canterbury also features localized pockets of exotic *Pinus radiata* D. Don forests, planted for both their windbreak and timber potentials. The earthworm fauna are all but absent in these modified habitats (pers. observation). However, three commonly encountered adult craneflies include *L. dicroithorax*, *L. tapleyi* and *L. zeylandiae*. The larvae are abundant at the interface of the H and mineral horizons, beneath accumulated litter; and may be as deep as 7cm below the mineral soil surface.

Analysis of gut smears stained with lactophenol cotton blue (Harris 1988) has confirmed these larvae have a fungivorous trophic status (unpublished data). However as substantial quantities of

mineral soil and other unidentified organic matter were also observed to be present, a fungivorous/geophagous status may be more appropriate. Johns (1980) described tipulid larvae as generalist feeders and subsurface browsers. The larvae are therefore potential regulators of fungal growth and may also facilitate the translocation of microbial material throughout the substrate via fecal exuviae, as has been described for earthworms (Devliegher and Verstraete 1997).

In the absence of both indigenous and introduced earthworms at the sampled horizons in these planted forests, it is interesting to speculate whether the larvae have a capacity to increase the total volume of pore space in soil, similar to that attributed to earthworms (Hoeksema and Jongerius 1959, Satchell 1967). Soil aeration is an important ecological process which facilitates gaseous exchange, a fundamental requirement for efficient plant root functioning (Baver and Farnsworth 1940, Bridge and Rixon 1976, Ruark *et al.* 1982, Theodorou *et al.* 1991). The creation of porous space in soil is also important for the invasion of soil by fungi (Otten *et al.* 1999).

The main intent of this chapter was to quantify the link between invertebrate abundance and the provision of an ecological process, soil porosity. It was hypothesized that the abundance of cranefly larvae would decrease under incremental biosolid applications, and limit their participation in soil physical processes. This is because biosolids were expected to physically and chemically modify the soil and litter habitat and limit the abundance of successive cranefly populations.

Experimental manipulation of the percentage cover of biosolids in field experiments identified a significant decrease in the abundance of cranefly larvae with incremental rates of biosolids application. However, a laboratory experiment designed to determine the effects of larval abundance on soil porosity, found larval abundance had no effect on soil pore size distribution and air filled porosity, at either of the two levels of soil bulk density tested (0.9 g cm^{-3} ρ_b and 1.1g cm^{-3} ρ_b) or at either of two soil matric potentials tested (-0.01 MPa and -0.05 MPa). These outcomes are discussed in relation to biodiversity, the historical changes in the regional fauna, the potential for differential effects on selected species and the provision of ecological services by larval tipulids in planted forests.

B. MATERIALS AND METHODS

1.Experimental design

The plot type, design, treatments and expected effects for the two experiments reported in this chapter are summarized in Table 5.1. The intention of the field-based manipulations was to demonstrate the effect of biosolids applications on demographic parameters of the cranefly larvae. The intention of the laboratory-based manipulation was to examine the capacity of cranefly larvae to influence soil physical characteristics.

Table 5.1 Plot type, experimental design, treatments applied and expected effects for Experiment 1A and 1B (caged and open plots) and Experiment 2 (soil columns).

PLOT TYPE AND DESIGN	TREATMENT	EXPECTED EFFECT
Experiment 1A Split plot design Field-based Paired incremental treatments in caged plots:	Control: Both secondary plots untreated	No effect on larval abundance
	Biosolids applied to one secondary plot. Adjacent secondary plot untreated	Decrease in abundance with incremental application rate in treated secondary plot. No effect on larval abundance in untreated adjacent secondary plot
Experiment 1B: Randomized block Field-based Single incremental treatments in open plots	Control: Main plot untreated	No effect on larval abundance
	Treatment: Biosolids applied to main plot	Decrease in abundance with incremental application rate
Experiment 2: Randomized block Laboratory-based	Control: Soil column Nil larvae ρ_b 0.9 g cm $^{-3}$ and 1.1g cm $^{-3}$ Ψ_m: -0.01 and -0.05 MPa	No effect on air-filled porosity
	Treatment: Soil column with 4 or 10 larvae ρ_b : 0.9 g cm $^{-3}$ and 1.1g cm $^{-3}$ Ψ_m: -0.01 and -0.05 MPa	An increase in air-filled porosity

2. Experiments 1A and 1B

Two individual experiments (1A and 1B) were conducted simultaneously at the same site, to determine whether incremental rates of biosolids application caused larval populations to decline. The research hypothesis for these experiments was "that the abundance of larval craneflies decreases under incremental biosolids applications". The experiments were conducted independently because they involved different levels of manipulation of both the larvae and the litter habitat.

Experiment 1A was designed to examine the effect of incremental biosolid application rates on the abundance of cranefly larvae in manipulated caged plots sited randomly between tree rows in a 21-year old *P. radiata* planted forest. Larval abundance within the cages was expected to

decrease linearly in relation to biosolid application rate. No difference was expected in the abundance of larvae in the untreated (control) secondary plots adjacent to each treated, secondary plot within a cage.

Experiment 1B was designed to examine the effect of incremental biosolids application rates on the abundance of cranefly larvae in unmanipulated open plots sited randomly between tree rows in a 21-year old *P. radiata* planted forest. Larval abundance was expected to decrease linearly in relation to biosolid application rate. The methodology for Experiments 1A and 1B are as follows:

2.1 Site selection and plot layout

During late winter (September), 1999, a transect survey of the larval craneflies was undertaken in a second rotation *Pinus radiata* stand which had been planted in 1978 at a rate of 1250 stems/ha (spacing 4 m x 2 and pruned and thinned to waste in 1985. A dense needle litter (up to 5cm deep) overlaid the mineral soil classified as a Lismore stony silt loam (D.S.I.R. 1968).

A metal grid (32 cm x 32 cm) was placed at 2 m intervals along 2 mid row transects each 20 m long. All litter plus mineral soil to a total depth of 10 cm from the surface, was removed and placed in a coarse (0.5 cm mesh) sieve. The mean density (±SD) of larvae retrieved from 20 samples was estimated to be 45 (±15) m^2. Larval density was four times higher than that recorded by Johns (1980) in *P. radiata* stands near Hanmer although densities of up to 148 m^2 were recorded at the same location under *Pinus nigra.*

An experimental area, of approx. 40 m x 100 m, at least 100m from the transect survey, was established in early spring (October 1999). Thirty-two mid row plots (each 2m x 1m) were pegged out. Twelve of these main plots were used for the caged trial and the remaining 20 plots for the open plot trial. Main plots for both trials were interspersed, at least 2m apart and located such that forest floor undulations and wind-thrown debris were avoided. The open plot experiment was conducted in conjunction with the caged plot experiment, as it was unknown whether reproductive behaviour (swarming, copulation) might be constrained by the cage.

2.1 Cage construction

Twelve wooden cages (internal dimensions 1m x 0.5 m x 0.5 m) were enclosed on five sides with taut green shadecloth (EnviroTex light knitted shadecloth 48% green / 52% black, Donaghy's Industries Ltd., Christchurch) leaving one long side open which was to be placed in contact with the mineral soil surface.

2.3 Plot preparation and placement of larvae for Experiment 1A

The caged plots were prepared by placing a wooden frame (0.5 m x 1 m) in the centre to form a primary plot and gently peeling back the litter area enclosed within the frame and placing the litter material, intact, onto a plastic sheet. The exposed humus and underlying mineral soil was removed (total depth of mineral soil to approx. 7 cm) and sieved (0.5 cm mesh) to remove all tipulid larvae. The sieved soil was returned to the plot and lightly compacted by hand. Larvae were temporarily stored in aerated plastic containers on damp filter paper. Additional larvae were taken from the litter in nearby forest beyond the experimental area.

Head capsule width and body lengths were measured in the laboratory from a subsample of 30 larvae. As more than 90% were judged to be third instar (Skerman 1953), it was expected that the sample was a good representation of the early summer emergent *L. dicroithorax*. Had the larvae been mixed species (early and late summer emergents), the caged plot experiment may have been compromised because species were mixed.

Within each primary plot, 2 adjacent secondary plots (each 32 cm x 32 cm) were marked out, each with an 18 cm median buffer and a 9cm perimeter buffer (Figure 5.1). Twenty-five randomly selected larvae were evenly distributed on the soil/humus mix in only one of each pair of secondary plots. The litter layer was carefully replaced over the larvae and a cage was placed over the top with the base at least 2 cm below the mineral soil surface. Plots were left undisturbed for 7 days prior to biosolids treatment.

2.4 Plot preparation for Experiment 1B

No manipulation of either the litter surface or the abundance of naturally occurring cranefly larvae was made. The biosolids were applied manually.

2.5 Biosolids collection and manual application

Freshly collected dewatered biosolids (Christchurch Wastewater Treatment Plant, Bromley) which had been stored in sealed plastic bags were applied manually to the experimental plots within 24 hours of collection. The application rates for both open and caged plots were Nil, 400, 800 and 1200 kg N/ha. The entire area of each open plot was treated. Only one of each pair of secondary plots in the caged experiment was treated. Treatments were applied to the caged plots by gently removing the cage and protecting the untreated adjacent secondary plot with a sheet of cardboard.

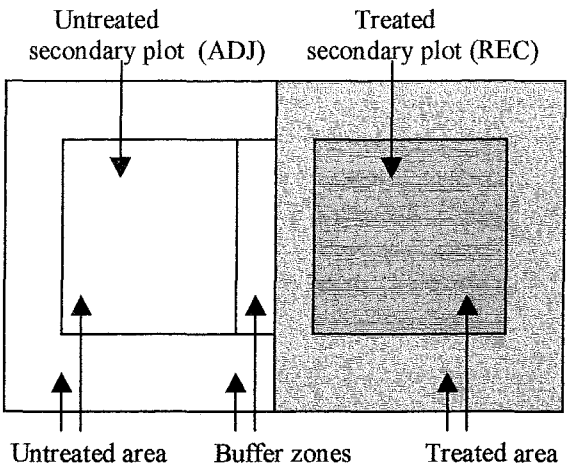

Figure 5.1. Diagram of the caged plot layout (Experiment 1A). The untreated (ADJ = adjacent) secondary plot was separated by an 18 cm median buffer from the treated (REC = receive) secondary plot. Secondary plots were surrounded by a 9 cm perimeter buffer. The cage was placed over the paired secondary plots and the base frame was excavated 2 cm into the mineral soil.

2.6 The time frame for Experiments 1A and 1B

Both caged and open plots were left undisturbed for 9 months, allowing for both the naturally-occurring (open plot) and manipulated (caged plot) larval cohort to develop. The larvae were expected to progress through the development succession of pupation, emergence, copulation and egg dispersal, egg filtration, development, pupation and the subsequent emergence of the second cohort in the soil. Cages were checked regularly for disturbance and to clear litter, which had accumulated on the upper surface.

2.7 The percentage cover of manually applied biosolids

The percentage cover of biosolids is dependent on the water content of the biosolids and the application system. To estimate the relationship between application rate and percentage cover, fresh dewatered biosolids were applied at rates equivalent to 400, 800 and 1200 kg N /ha., to 6 replicate 1 m x 1 m plots (3 plots per treatment). The plots were on a flat surface, which had been covered with plastic sheeting. A digital camera (Olympus CAMEDIN C-840L) was used to photograph each replicate from directly overhead. Photographs were analyzed using Metamorph image analysis software (Universal Imaging Corporation, 1995) and the average of three readings per replicate used as an estimate of percentage cover of biosolids for each application rate.

2.8 Retrieval of larvae

The larvae present in open and caged plots were retrieved in July 2000 (Figures 5.2 and 5.3). The timing ensured these early emergent larvae were at least the third instar, and had not begun to

pupate. All larvae occurring in both the untreated and treated, secondary caged plots were removed. A 32cm x 32cm grid was randomly placed twice in each open plot and the mean abundance (±SD) of larvae calculated. Larvae from all replicates were kept separate and taken to the laboratory. All larvae were subsequently examined under high power; the similarity of headcapsule width and body length measurements suggested they were most probably *L. dicroithorax*.

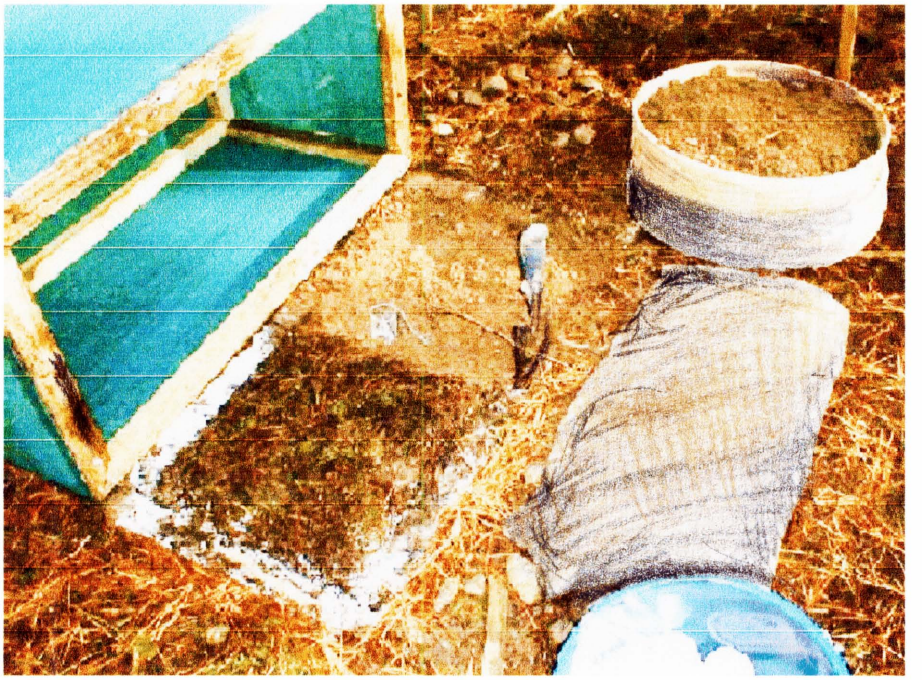

Figure 5.2 Experiment 1A. Retrieval of larvae from paired secondary plots in caged experiment, using a 32x 32 cm grid.

Figure 5.2. Experiment 1B. Removal of cranefly larvae from an untreated open plot, using a 32 x 32 cm grid. Each plot was marked with four corner stakes.

3. Experiment 2

This second experiment was designed to examine the effect of larval abundance on soil bulk density and soil physical properties. Biosolids were not used in any part of this experiment. The research hypothesis was "that cranefly larvae increase the air-filled porosity of the soil." It was expected that incremental larval abundance would have a positive treatment effect on soil pore size distribution and air filled porosity.

3.1 The soil

Lismore stony silt loam soil was obtained from several forest sites in the Dunsandel-Hororata locality during March 2001. The mineral soil (to a depth of 7 cm) and the H horizon were sieved (0.5 cm and 0.2mm mesh) to homogenize the sample, remove large debris and extract cranefly larvae. Soil was stored at 4°C in large sealed plastic bags in plastic bins covered with lids prior to use. I had previously found larvae to be easily maintained in similarly-treated soils for many weeks at a time, with all animals maintaining good vigour (a strong negative phototaxic response) and acceptable growth rates comparable with those in the field. Bags were opened regularly to permit aeration. Five weighed subsamples were taken from the stored soil and oven dried for 24 hours at 105°C to measure soil moisture content (McLaren and Cameron 1996). The estimated

soil moisture content of 8.5% was used to calculate the amount of field-moist soil required to pack the soil columns at the specified soil bulk densities (ρ_b). A general methodology (Taylor 1972) was used to estimate the mean particle density (ρ_p) of the soil samples. This was found to be was 2.027 ρ_p (±0.125).

3.2 Preparation of the soil columns

PVC pipe (50 mm internal diam.) was machine-cut into three different lengths (i) 10 mm rims (ii) 70 mm cylinders for soil (volume 137.5 cm^3) (iii) 500 mm cylinders for sand (Figure 5.4). Field moist soil was packed into each of the seventy two, 70 mm columns. The weight of soil used for the lower bulk density (0.9 g cm^{-3} ρ_b) was 134.3 g and for the higher bulk density (1.1 g cm^{-3} ρ_b), 164.1 g. A layered packing technique was used to achieve an even distribution of bulk densities through the soil column (Penfold 1998). The 500 mm cylinders were sealed at one end with two layers of Whatman 1 filter paper secured with a rubber band, loosely packed with fine river sand and placed on draining racks in plastic trays. Water was added to the surface of each sand-filled column until it was free-draining. This primed the sand in order to guarantee free drainage of water through each 70 mm soil-filled column.

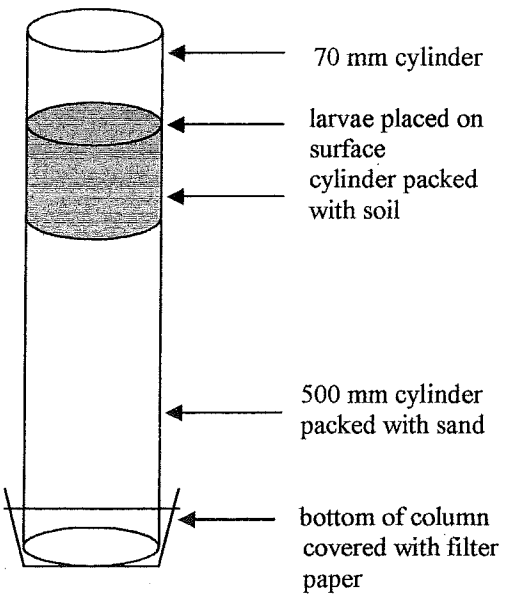

Figure 5.4 Experiment 2. Diagram of soil column setup. To ensure the soil in each replicate column was free draining, columns were stood on a plastic grid in a draining tray for the duration of the treatment period.

Extra soil was then added to the sand surface of each 500mm column, flush with the rim, and gently integrated with the sand, to avoid a sudden changes in filtration rate. A 500mm sand-filled column was secured underneath each 70mm soil-filled column using heavy-duty waterproof tape. An additional 70 mm empty cylinder was secured to the top of each soil-filled cylinder to complete the experimental unit. All cylinders were placed into a controlled environment cabinet at 12°C. Each column received 200 ml H_20 on the first day and was checked to ensure free drainage. The columns were left for 7 days to equilibrate.

3.3 The larvae: preparation and experimentation

Cranefly larvae acclimatized to the controlled environment conditions (80% relative humidity, 14/12 light/dark regime, and 12°C temperature) were used in the experiment. Columns were treated in replicate groups of 12, such that larvae were evenly distributed on the surface of a soil-filled column at either of three levels of abundance (0, 4 and 10 larvae), such that the total period of interaction between the larvae and the soil in the controlled environment cabinet was 30 days for each column. Larval density treatments were selected arbitrarily.

3.4 Quantifying air filled porosity and soil water content

Replicates were removed from the controlled environment cabinet, dismantled, and small irregularities in both the upper and lower soil surfaces were amended with a little extra soil to ensure the soil-packed columns contained the maximum volume of soil. A 10 mm rim was secured to the top surface of each column with PVC tape (Figure 5.5). Water was added to the column to the level of the rim and soil-filled columns were placed on a sheet of Whatman 1 filter paper on a pre-soaked (24 hours) 1 MPa ceramic plate in a pressure plate apparatus (Eijkelkamp, Agrisearch Equipment, Giesbeek, The Netherlands), set initially at a soil matric potential (Ψ_m) of –0.01 MPa. The columns were left to equilibrate (average of 5 days). At equilibration, water no longer dripped from the outflow.

Columns were removed from the apparatus, weighed, placed on filter paper on a pre soaked 1 MPa ceramic plate in an adjacent pressure plate apparatus at a soil matric potential (Ψ_m) of -0.05 MPa and again left to equilibrate (average 19 days). The columns were removed, dismantled and the individual units weighed. The soil was crumbled and placed in paper bags and oven dried for 24 hours at 105°C before being re-weighed. The dry weight of soil was used to accurately calculate the bulk density for each replicate.

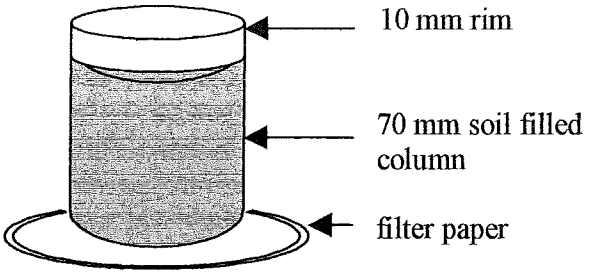

Figure 5.5 Experiment 2. The soil column arrangement with the additional 10 mm rim taped to each column prior to processing in the pressure plate apparatus. A sheet of filter paper was placed under each column before placement on the pre-soaked ceramic plate to limit the movement of fine particles.

3.5 Equations

The definitions and formulae used in calculations (McLaren and Cameron 1996) are as follows:

The gravimetric water content (θ_g) is the ratio of the mass of water to the mass of solids and is given by the equation

$$\theta g = \frac{Ww}{Ws}$$

where W_w is the weight of the water and W_s is the weight of the soil.

The volumetric water content (θ_v) is the ratio of the volume of water to the total volume of the soil and is given by the equation

$$\theta v = \frac{Vw}{Vs}$$

where V_w is the volume of the water and V_s is the volume of the soil.

Soil dry bulk density (ρ_b) (g cm^{-3}) is the ratio of the mass of dry soil to the total volume of the soil and is given by the equation

$$\rho b = \frac{Ws}{Vs}$$

where W_s is the weight of the soil (g cm^{-3}) and V_s is the volume of the soil (cm^{-3})

The particle density (ρ_p) of the soil is the ratio of the total mass of solids to the volume of solid material and is given by the equation

$$\rho_p = \frac{Ms}{Vs}$$

where M_s is the mass of solids and V_s is the volume of solids.
By substitution, the volumetric water content (θ_v) is

$$\theta v = \frac{Ww \times \rho_b}{Ws}$$

where W_w is the weight of the water, ρ_b is the bulk density of the soil and W_s is the weight of the soil. And by further substitution,

$$\theta v = \theta g \rho_b$$

The total porosity (ε) of the soil is the ratio of the volume of pores to the total volume of soil and is given by:

$$\varepsilon = 1 - \frac{\rho_b}{\rho_s}$$

where ρ_b is the bulk density of the soil and ρ_s is the particle density of the soil.
The air filled porosity (ε_a) at water content θ_v is therefore given as

$$\varepsilon_a = \varepsilon - \theta_v$$

4. Analysis

A split plot design was used for the caged plot experiment. Caged plot data were analyzed using the General Linear Model Procedure of SAS (SAS/STATS 96) followed by a test to establish linearity of treatments. A random block design was used for the open plot experiment and analyzed using one way ANOVA with equal sample size followed by simple linear regression. A line of best fit was fitted to the data. Levene's test of equal variance was not violated for either caged or open plots.

The replicated column experiment was analyzed using Two way ANOVA with equal sample size, followed by Tukey's Multiple Comparison Test (Graphpad PRISM).

C. RESULTS

1. Percentage cover

The percentage cover of manually applied biosolids increased with application rate, however, the effect was not linear (Figure 5.6). Percentage cover at the three application rates was 55% (400 kg N/ha), 72% (800 kg N/ha) and 88% (1200 kg N/ha). The effect from 0-400 kg N/ha was much greater than the effect between 800 and 1200 kg N/ha. This could have been due to successive applications of biosolids "clinging" to the top and sides of biosolids already on the ground, thus forming many hump-shaped mounds, rather than scattering freely across the plot area. It is unknown whether this graph can be applied to machine-spread biosolids.

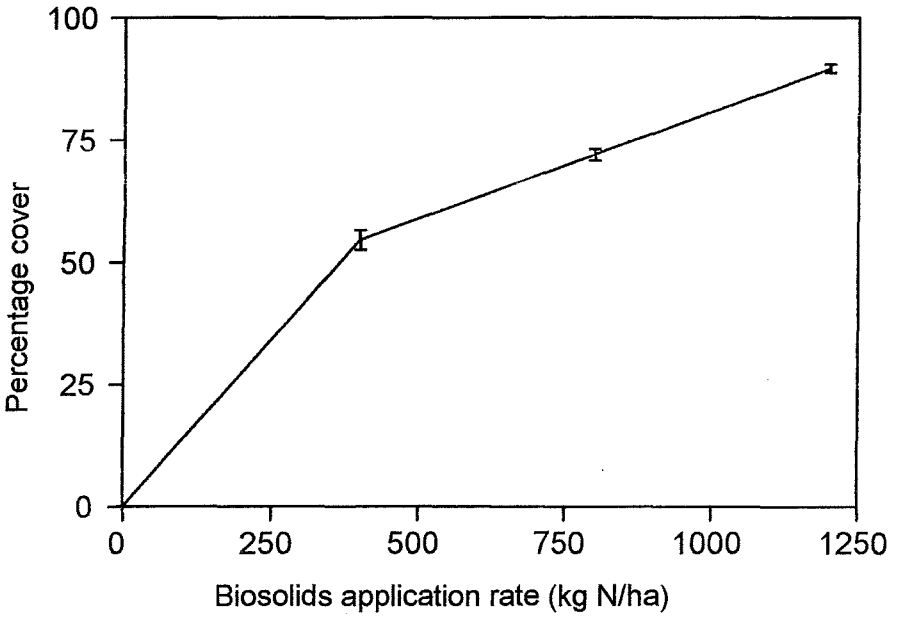

Figure 5.6 The percentage cover of manually applied biosolids at the three application rates 400, 800 and 1200 kg N/ha. Values are the mean (±SD) of 3 values for each treatment rate.

2. Experiment 1 A: Caged plots

The application of biosolids significantly affected the mean abundance of larvae under all rates of biosolid application in caged plots ($P < 0.001$)(Table 5.2, Figure5.7). The difference in mean larval abundance between 0 and 400 kg N/ha application rates was far greater than the differences between 800 and 1200 kg N/ha. Significant differences were also found in the mean abundance of larvae in untreated secondary plots (ADJ) at incremental application rates ($P < 0.001$). A

significant interaction ($P < 0.05$) was found between the biosolid-treated secondary plots and adjacent, untreated, secondary plots. The mean abundance of larvae in untreated secondary plots (ADJ) adjacent to treated secondary plots (REC) decreased linearly with increasing application rates ($r^2 = 0.6505$, $P < 0.001$).

Table 5.2. Experiment 1A. ANOVA summary statistics for mean larval abundance in caged plots data

Source	df	SS	Mean Square	F	P value
BLOCK(B)	2	1.65	0.82	3.97	0.063
TREATMENT (T)	3	32.09	10.69	18.77	0.001
B*T	6	3.42	0.57	2.74	0.094
RECEIVE (R)	1	19.01	19.01	91.41	0.001
T*R	3	5.81	1.94	9.32	0.01
LINEAR TREAT	1	30.79	30.79	54.03	0.001
QUAD TREAT	1	0.49	0.49	0.87	0.386

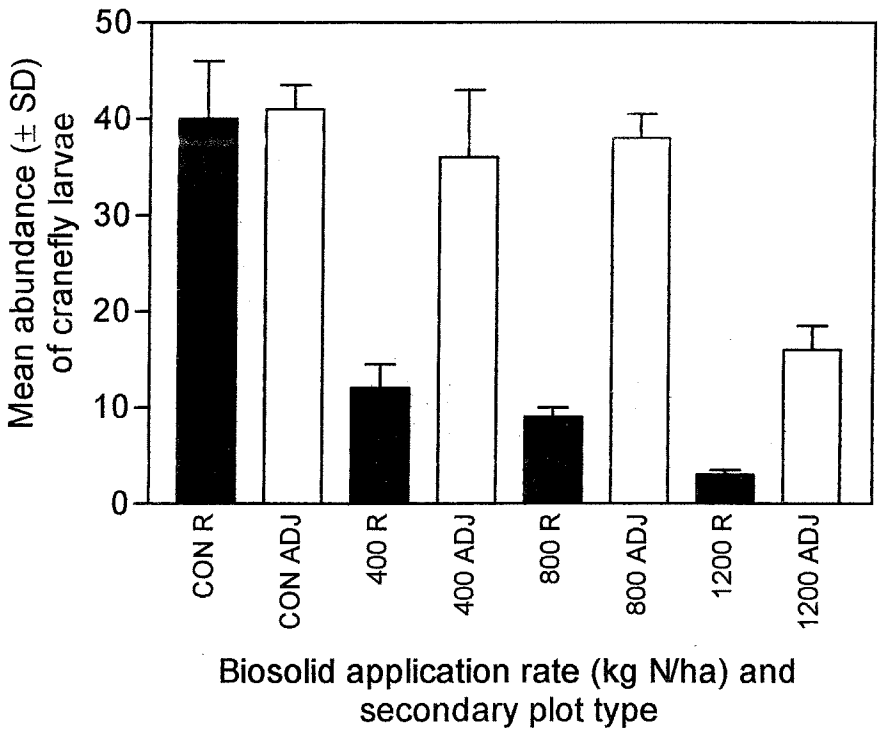

Figure 5.7. Experiment 1A. The mean abundance (+SD) of larval craneflies in caged secondary plots receiving (R) biosolid treatments or adjacent (ADJ) to a secondary plot receiving biosolids. Biosolid treatments were equivalent to Nil, 400, 800 and 1200 kg N/ha. Control (CON R and CON ADJ) plots did not receive biosolids.

3. Experiment 1B: Open plots

Mean larval abundance decreased significantly in open plots under incremental biosolids application rates (ANOVA, $F = 76.92$, $P < 0.0001$) (Table 5.3). The decrease in mean larval

abundance was greater between 0 and 400 kg N/ha than between 800 and 1200 kg N/ha. Simple linear regression identified a significant and negative linear relationship between larval abundance and incremental biosolid application rate ($r^2 = 0.8439$, $P < 0.0001$) (Figure 5.8).

Table 5.3 Experiment 1B. ANOVA summary statistics for mean larval abundance in open plots

Source	df	SS	MS	F	P value
TREATMENT	3	1927	642.3	76.92	0.0001
RESIDUAL	16	133.6	8.35		
TOTAL	19	2061			

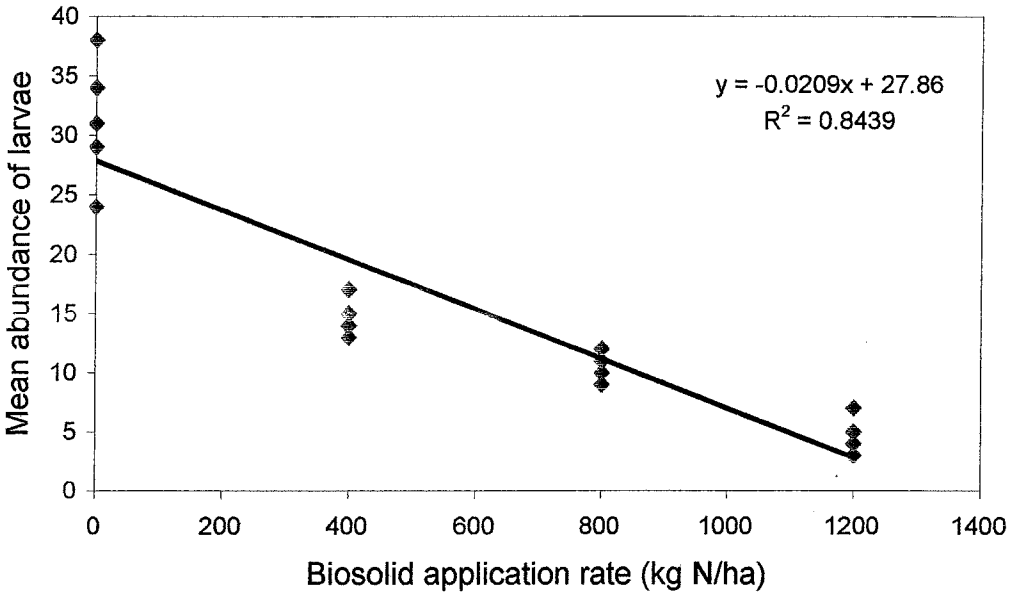

Figure 5.8 Experiment 1B. Relationship between the mean abundance of larval craneflies in open plots receiving biosolids treatments at application rates of Nil, 400, 800 and 1200 kg N/ha. N = 5. (Note in the treated open plots there were instances of the same abundance value occurring, for which symbols are overprinted in the graph)

4. Experiment 2

As was expected, air filled porosity (ε_a) at soil matric potential -0.01MPa was significantly lower in the high bulk density treatment ($\rho_b = 1.1$g cm^{-3}) than in the low bulk density treatment ($\rho_b = 0.9$ g cm^{-3}) ($P < 0.0001$). Similarly, air filled porosity (ε_a) at soil matric potential -0.05 MPa was significantly lower in the high bulk density treatment ($\rho_b = 1.1$g cm^{-3}) than in the low bulk density treatment ($\rho_b = 0.9$ g cm^{-3}) ($P < 0.0001$). Larval abundance had no effect on air filled porosity at either of the two bulk density treatments at soil matric potential -0.05MPa. There was evidence of a weak interaction (larval abundance*bulk density) at -0.05 MPa (Table 5.5, Figure 5.9).

Table 5.4 Experiment 2. Two way ANOVA Summary statistics for air filled porosity at three levels of larval abundance and two levels of bulk density calculated at -0.01MPa.

Source	df	SS	MS	F	P value
Interaction	2	0.000475	0.0002378	0.2242	0.7998
Larval abundance	2	0.003667	0.001834	1.729	0.1854
Bulk density	1	0.5601	0.5601	528	$P < 0.0001$
Residual	66	0.07001	0.001061		
Total	71				

Table 5.5 experiment 2. Two way ANOVA Summary statistics for air filled porosity at three levels of larval abundance and two levels of bulk density calculated at -0.05MPa.

Source	df	SS	MS	F	P value
Interaction	2	0.003232	0.001616	3.244	0.0453
Larval abundance	2	0.001838	0.0009189	1.845	0.1661
Bulk density	1	0.3919	0.3919	786.8	$P < 0.0001$
Residual	66	0.03288	0.0004981		
Total	71				

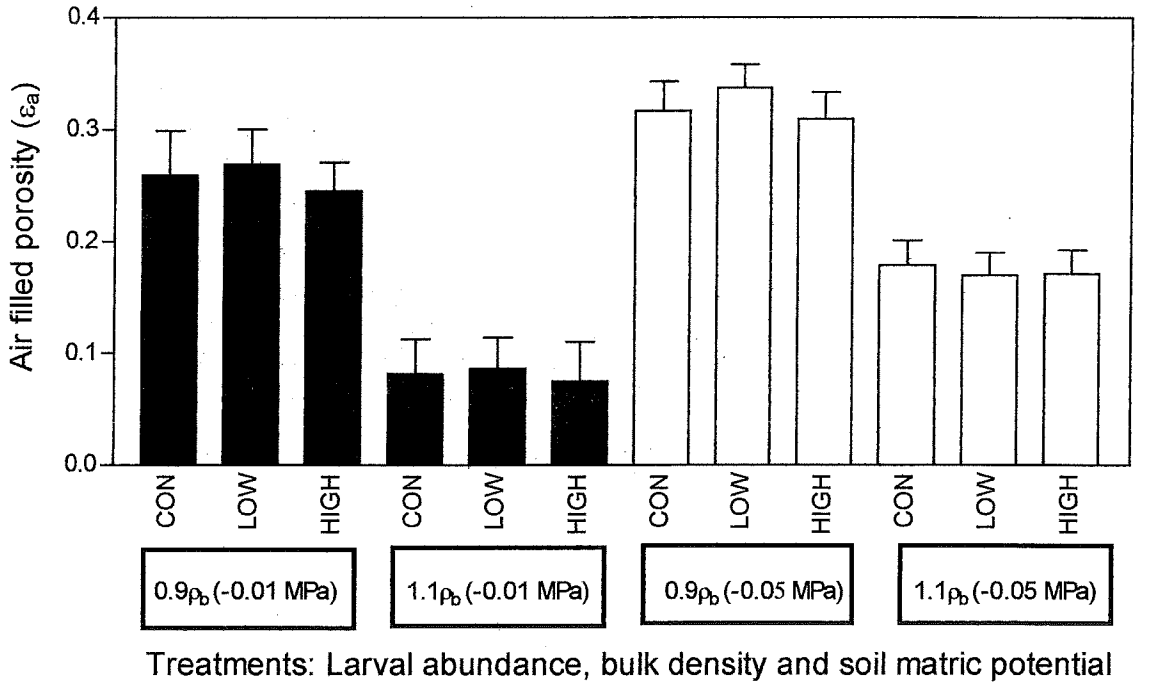

Figure 5.9. Experiment 2. Mean air-filled porosity (ε_a) of soil columns at two bulk densities (ρ_b) 0.9 g.cm^{-3} and 1.1 g.cm^{-3}, three levels of larval abundance CON (Nil), LOW (4) and HIGH (12) and two soil matric potentials (Ψ_m) (-0.01 MPa and -0.05 MPa). The boxed bulk density and soil matric treatments refer to the set of three larval abundance levels directly above each box.

The volumetric water content (θ_v) of the soil was also significantly lower in the high bulk density treatment (ρ_b = 1.1g cm $^{-3}$) than in the low bulk density treatment (ρ_b = 0.9 g cm^{-3}) at soil matric potential -0.01MPa ($P < 0.0001$) (Table 5.6, Figure 5.10). Similarly, the volumetric water content (θ_v) of the soil was significantly lower in the high bulk density treatment than in the low bulk density treatment at soil matric potential -0.05MPa ($P < 0.0001$) (Table 5.7, Figure 5.10).

Table 5.6 Experiment 2. Two way ANOVA Summary statistics for volumetric water content at three levels of larval abundance and two levels of bulk density calculated at -0.01MPa.

Source	df	SS	MS	F	P value
Interaction	2	0.0005722	0.0002861	0.2812	0.7558
Larval abundance	2	0.001642	0.000821	0.8069	0.4506
Bulk density	1	0.1502	0.1502	147.6	P < 0.0001
Residual	66	0.06715	0.0001017		
Total	71				

Table 5.7 Experiment 2. Two way ANOVA Summary statistics for volumetric water content at three levels of larval abundance and two levels of bulk density calculated at -0.05MPa.

Source	df	SS	MS	F	P value
Interaction	2	0.002203	0.001102	2.615	0.0808
Larval abundance	2	0.001294	0.0006469	1.535	0.223
Bulk density	1	0.079996	0.07996	1.89.8	P < 0.0001
Residual	66	0.02781	0.0004213		
Total	71				

At the higher bulk density of 1.1 g. cm $^{-3}$, the air-filled porosity was less than 0.10 v/v. There was no effect of larval abundance on soil pore size distribution and air filled porosity at either bulk density or at the two soil matric potentials (Ψ_m) (-0.01 and -0.05 MPa).

The difference in θ_v between –0.01 kPa and –0.05 kPa soil matric potential was greater at the high (ρ_b 1.1) than at the low (ρ_b 0.9) bulk density. This confirms that the process of increasing ρ_b reduces the size of air-filled pores, so that they become water-filled, with a concomitant increase in the proportion of water-filled pores. Therefore, the effect of increasing bulk density is the same as having a finer textured soil. During the experimental period in the controlled environment cabinet, the volumetric soil water content for each bulk density treatment was 44 % (0.9 g cm^{-3} ρ_b) and 64 % (1.1 g cm^{-3} ρ_b), corresponding to 55 % and 46 % porosity respectively.

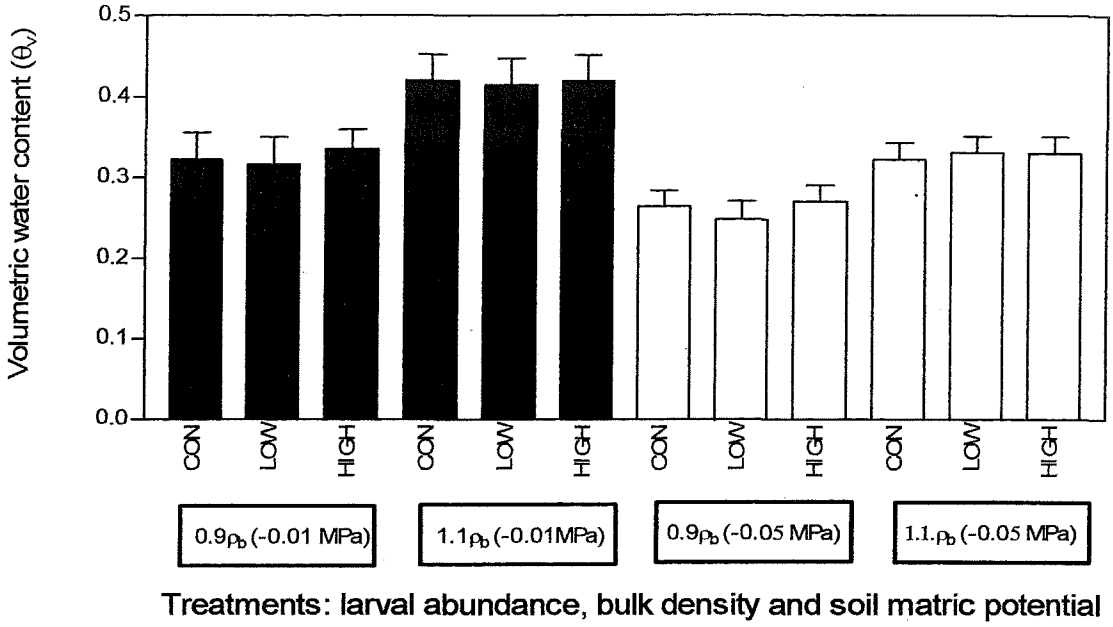

Figure 5.10 Experiment 2. Mean volumetric water content (θ_v) of soil columns at bulk densities (ρ_b) 0.9 g.cm^{-3} and 1.1 g.cm^{-3}, three levels of larval abundance CON (Nil), LOW (4) and HIGH (12) and two soil matric potentials (-0.01 MPa and -0.05 MPa). The boxed bulk density and soil matric treatment levels refer to the set of three larval abundance levels directly above each box

No larval mortality was observed during the treatment period in the controlled environment cabinet. However, > 80% of the larvae died during the two equilibration phases in the pressure plate apparatus. Thus, the larvae were assumed to have had little or no effect on the physical properties of the soil during the periods of equilibration.

D. DISCUSSION

The physical effect of the percentage cover of biosolids on the surface of the forest floor

The non-linear relationship between percentage cover and manually applied incremental biosolids application rate indicated that a thicker (more dense) coverage, rather than a wider spread, occurred with increasing rates of application (Figure 5.6). The effect of a steep ramp in percentage cover between Nil and 400 kg N/ha and a less acute slope between 800 and 1200 kg N/ha treatment rates, was found to have a mirror effect on larval abundance in the caged field experiments. There was a steep decline in larval abundance in caged secondary plots receiving 400 kg N/ha, compared with caged secondary plots receiving Nil biosolids; the decline in larval abundance between the 800 and 1200 kg N/ha treatments was notably less acute.

These results confirm the predictions that larval abundance would decline under incremental biosolids application rates. However, care is cautioned in direct extrapolation of effects to other age classes, and also where biosolids are applied mechanically. In the field, following large-scale applications by a purpose-built machine, biosolid material applied to pre-canopy age classes was observed to dry into irregular postcard-sized (and smaller) patches of persistent cardboard-like crust, with edges curling characteristically upwards. Substantial rainfall occurring shortly after application, was observed to smooth the patch surface and encourage a lateral spread. Thus, the actual percentage cover *in situ* is likely to be mediated by local climate, stand architecture and the peculiarities of the application system. A thick crust is likely to have consequences for microbial and soil respiration, as the free exchange of gases could be impaired, creating localized anoxia and favouring anaerobic bacteria. Lateral and horizontal mobility of invertebrates within the surface litter may also be limited, restricting the suitability of the habitat for some species.

The effect of biosolids applications on larval abundance in caged and open plots

Both the caged and open plot experiments clearly demonstrated a significant decrease in the abundance of larval craneflies in the pine forest litter under all rates of biosolids application (Figures 5.7 and 5.8). The decrease in larval abundance was attributed to the dewatered biosolids forming a physical barrier to the passive, vertical filtration of broadcast eggs (or the newly hatched larvae) through the litter to the H horizon and mineral soil interface. Fertilized eggs may hatch within three days (Meats 1972) and the retention of eggs (or newly hatched larvae) on the surface increases the risks of desiccation, flooding and fungal attack. A significant decrease in the survival and development of eggs of *T. oleracea* and *T. paludosa* broadcast over pools of stagnant water lying on the soil surface has been demonstrated (Meats 1972).

Eggs retained above the litter may also be exposed to predation by Opiliones (harvestmen) (Sergeva 1999) harpaline (Coleoptera: Carabidae) and staphlinid (Coleoptera: Staphylinidae) beetles (Lukasiewicz 1996, Hu and Frank 1997). These predatory beetles are common in the mid Canterbury planted forest habitat (refer to Chapters 3 and 4). The cranefly larvae in forest and woodland habitats are also prone to predation by birds; potential predators in the mid-Canterbury forests include magpies, pheasants, quail and fantails (pers. observation).

The caged treatments may have protected the larvae from predation. Similarly, normal swarming and courtship behaviour by emergent adults may also have been restricted. This was a key reason for incorporation of open plots into the experimental design. Had there been high variability in mean larval abundance between Experiments 1A and 1B under similar biosolids application rates,

a cage effect may have been inferred. However, despite potentially different initial larval abundances between the unmanipulated open and "seeded" caged plots, the variability in larval abundance between the same biosolids treatment rates in both open and caged plots was very low. This outcome suggests it is unlikely a cage effect confounded the experiment. Furthermore, had lateral migration of larvae occurred out of the cages (they were set into the soil at less than their observed potential vertical range in the soil), or across plots within cages, then, if all else were equal, other larvae would have migrated inwards. This uncontrolled-for variable may have confounded the results. However, the biosolids effect was so profound that lateral migratory effects, either in or out, failed to obscure the outcomes.

It was thought that the biosolids would act as an attractant to the female, rather than a deterrent. This is because the gut of the adult cranefly atrophies during pupation. Cranefly adults are susceptible to desiccation and although they do not feed, they may take in liquids, such as condensate on surfaces (Freeman 1967). Thus, an uneven biosolids surface may contain small pockets of moisture from condensation or precipitation, which may be preferentially sought by the female. Furthermore, all reports in the literature characterize the family as having short-lived, often crepuscular, adults (Barnes 1937) which predominantly swarm after sunset and tend to mate near to the ground (Lewis and Taylor 1965). Following copulation, female tipulids have been shown to favour damp, cool, still, areas, to disperse eggs by broadcasting, in locations where air turbulence and convection are minimal (Service 1973, Rief 1996). The biological traits of the adult tend to favour dispersal over the biosolids. If this occurred, then passive filtration of the eggs into the litter was likely to have been influenced by the percentage cover and application rate of the biosolids.

The interaction between treated and adjacent, untreated secondary plots

The significant interaction between treated and untreated pairs of secondary plots (ADJ and REC) in the caged experiment is difficult to explain. Incremental application rates of biosolids applied to the treated secondary plots may have been responsible for a chemical effect leading to the stepwise decline in larval abundance in untreated adjacent secondary plots. The lateral movement of nutrient-rich leachates from the treated to the untreated plots may have affected microbial activity and fungal growth. This could happen in one of two ways. Either (i) lateral leaching of nutrients resulted in the stimulation of fungal growth, thus providing larvae with abundant resources, or; (ii) lateral leaching of nutrients stimulated fungal or microbial growth which

inhibited larval growth. If the first explanation was true, the stepwise decline in larval abundance in relation to incremental application rates is unlikely to have occurred.

The selective impact of biosolid applications on cranefly species

Biosolid applications may impact more strongly on some species of cranefly than others. For example, small-scale habitat heterogeneity contributes to the often-patchy distribution of larvae in the forest litter and larval clumping may be magnified by the idiosyncratic physiology of some species (Freeman 1967). Unpublished field observations by P.M. Johns have confirmed that the male *L. zeylandiae* awaits the delayed emergence of the brachypterous females at the litter surface and mating can occur within minutes of their emergence. The female either expels the fertilized eggs at the emergence site, or, alternatively, flies *in copulo* with the male, enabling eggs to be broadcast further afield. Therefore, in localized areas of the forest where biosolids form a barrier over the litter surface, it is most likely that abundance *of L. zeylandiae* would be compromised.

The effect of larval abundance on soil air-filled porosity

Within the experimental time frame of 30 days, the cranefly larvae did not contribute to a change in pore size distribution in the soil (Figures 5.9 and 5.10). The capacity of the soil invertebrate fauna to contribute to soil remediation, where bulk density was a limiting factor, would have been seen as the provision of a beneficial ecological process. The experimental methodology utilized an upper limit of 1.1 ρ_b, in which it was demonstrated that the air-filled porosity was less than 0.10 v/v. At that level of soil restraint, root elongation is restricted and plant growth reduced (Baver and Farnsworth 1940, Sands and Bowen 1978, Penfold 1998, Zou 1999). In forest stands, soil disturbance from repeated passes of heavy machinery and the removal of litter can contribute to elevated soil bulk density (Skinner *et al.* 1989).

Although the experimental conditions in the laboratory could not replicate the forest situation, the results may have been confounded by the inhibition of normal tipulid larval activity. This could be due to a variety of abiotic and biotic factors. Of these, soil moisture was a factor unlikely to have limited larval activity. However, reduced aeration resulting from compaction and the resultant low air and high soil strength might have reduced larval activity. Perhaps if larva do improve aeration and decompact soils, they can only do so if they first become established in relatively non-compact aerated soils and move from there into more compacted soils. The volumetric water content of the soil (44% at 0.9 g cm^{-3} ρ_b and 64% at 1.1 g cm^{-3} ρ_b) corresponded to porosity values of 55% and 46% respectively. These porosity values compare favourably with

typical New Zealand soils, where 53-57% is standard for a sandy loam/silt loam and 37-47% is standard for a silt loam/silt clay loam (McLaren and Cameron 1996).

The selected bulk densities may have provided an unrealistic habitat for larvae to function within. Larvae were previously shown to require a loosely structured substrate for passive filtration of eggs following broadcast dispersal by the female. Eggs landing on substrate which is closely packed are unlikely to successfully penetrate to the subsoil, without being either desiccated or preyed upon. The experimental substrate was hard-packed and smooth. Larvae encountering such conditions may never progress from the eggs stage. Selective pressures may therefore be acting to limit larval distribution in unfavourable microhabitats.

The selected bulk densities may have provided an unrealistic habitat for the measurement of larval activity as an individual species. The experiment assumed the larvae, alone, were able to alter soil structure. The generation of porous space may, however, be a cumulative effect, reliant on the interaction of two or more trophic groups.

The experimental results do not support casual field and laboratory observations. Larvae have been observed to actively and rapidly burrow into the soil in a negative phototaxic, response and it is not uncommon to find larvae to depths of 7 cm in the mineral horizon of the forest soils (Chapter Three). Furthermore, when a number of larvae were maintained for several weeks in a damp, soil/litter mix in a container in the laboratory, substantial tunneling and burrowing was noted.

It is most likely that the burrowing capacity of cranefly larvae involves lateral rather than vertical movement, which is similar to the indigenous surface feeding earthworms (Megascolecidae) (Lee 1959). Lateral tunneling and dispersion is typical of fauna which have evolved in a forest ecosystem, where a shallow litter overlies mineral soil, as is typical for the xeromorphic yellow-grey earths of the Canterbury Plains (Lee 1959, D.S.I.R. 1968). For these reasons it is suggested that the experiment may have been compromised by its short duration (30 days) as this had little relationship with the actual time spent by the larvae under natural field conditions (11 months).

It is also possible that a different soil type may have invoked a different response. Lismore silt loam is a lightly textured soil (> 75% silt, < 50% sand, < 25% clay), which has a naturally low bulk density (D.S.I.R. 1968). Whilst invertebrate movement in the soil may have created porous space, displacement of particles brought about by horizontal or vertical movement may only be temporary, with the voids being readily filled and even compacted, as the animal shifts.

Therefore, under some conditions, invertebrate activity may only be responsible for the redistribution of pore space, rather than an increase in the total soil pores (van Rhee 1969).

In view of the experimental results and given the relative abundance of larvae at the interface of the H and mineral soil horizons (as compared with a less regular occurrence at greater depths) the larvae must be considered essentially shallow surface feeders, which are not directly associated with a capacity to aerate soils.

Conclusions

Biosolids applied to the forest floor of *P. radiata* planted forests in mid Canterbury are likely to reduce the abundance of larval craneflies. Although the manual application of biosolids may not exactly mirror the percentage cover achieved by mechanical methods, the overall effect of biosolids, even at the lowest application rate tested (400 kg N/ha), resulted in a greater than 50% reduction in larval abundance. Of the three indigenous cranefly species known from these forests, *Leptotarsus zeylandiae* is most probably at greatest risk from biosolid applications.

The lack of convincing linkage between larval abundance and their capacity to contribute to soil aeration under laboratory conditions may be due to difficulties in establishing a suitable and sensitive methodology. The shallow feeding fungivores may have little influence on soil physical structure at the microhabitat scale. They are highly unlikely to provide an ecological service (at a very fine scale) for site remediation of soils following mechanical compaction. Ultimately, their ecological value may lie more directly with their mode of feeding, which bears comparison with some earthworm fauna, and is therefore likely to facilitate the regulation of fungal biomass and the translocation of microbial material in the forest. In doing so, they contribute to the recycling of soil nutrients important for plant growth.

These initial field-based investigations suggest that, at least in the short term, the application of biosolids at the lowest rates may diminish the ability of the exotic pine forest to sustain selected endemic dipterans. That may well have a negative impact on local biodiversity and ecosystem function.

CHAPTER SIX

THE LARVAL CRANEFLY *LEPTOTARSUS* (DIPTERA:TIPULIDAE) AS A BIOINDICATOR OF HEAVY METAL CONTAMINATION

A. INTRODUCTION

The development of acceptable limits for metal contaminants in soil is based on the ecological risk to a specified fraction of invertebrate species. In order to establish guideline limits, suitable invertebrate test species are subjected to a variety of contamination regimes. These toxicity tests define proportional responses, which are then calibrated against other published outcomes, resulting in a collective guideline for limit levels.

Guidelines adopted by regulatory authorities are generally set such that there is a 90% certainty that organisms growing in the soil are protected (Will and Suter 1994). However, such guidelines have been criticized as being too simplistic, as they often have little bearing on the availability of the metal to the resident biota (Peijnenburg *et al.* 1999). The corollary to this argument is that metal concentrations in the biota may only partially reflect environmental pollution levels anyway.

The indicator biota commonly used to examine the toxicity of specific contaminants in controlled environments under standardized conditions includes the Collembola, Lumbricidae and Isopoda. However, a high level of variability in the relative sensitivities of invertebrate species to heavy metals has been documented (Hopkin 1990, Morgan and Morgan 1991, Ecobichon 1992, Tomlin 1992, van Straalen and Bergema 1995, Wilczek and Migula 1996, Gräff *et al.* 1997). Therefore, enhanced confidence in existing guidelines for soil contaminants can only be gained by examining the demographic responses of the widest variety of ecologically relevant invertebrate species.

One group, the Diptera, have been recently promoted as potential indicators in ecological investigations, because of their wide distribution and close association with the soil of many species, particularly at the larval stage (Frouz 1999). The soil-dwelling larvae include phytophagous, fungivorous, detritovorous and geophagic species (Perel *et al.* 1971, Johns *et al.* 1980, Vlug and Harrewijn 1994, Rief 1996). These larvae provide an ideal vehicle to examine the relative toxicity of soil contaminants, as feeding behaviour variably involves the ingestion of contaminants dissolved in soil pore water, accumulated in fungal rhizomorphs and sporocarps,

bound to organic particles or coating mineral particles (Hopkin 1989, Roth 1992, Gräff *et al.* 1997).

Soil-dwelling larvae may provide a more holistic response to chronic contamination of the habitat, than the more commonly used gastropods. This is because tests using gastropods generally utilize pre-selected food substrates, which have been soaked in specific contaminants (Marigomez *et al.* 1986, Dallinger 1994, Triebskorn and Kohler 1996). Tests utilizing soil-dwelling larvae depend on the larvae feeding from material already existing in the contaminated substrate. Their responses to specific soil contaminants may therefore incorporate a response to soil biological activity, reflecting effects at the bottom end of the food chain.

Some soil-dwelling larvae have a tough outer skin, which affords them protection from desiccation (Freeman 1967, Young and Koenig 2000). These species are less likely to accumulate soil contaminants by passive uptake across a moist cuticular outer membrane, as has been found for earthworms (van Gestel and Ma 1988, Rundgren and Nilsson 1997). Earthworms have been used widely in chronic toxicity tests and their relative sensitivities to metals form the basis for metal limit level guidelines in soils Cuticular uptake can heighten the relative sensitivity of a species to selected concentrations of metal contaminants in the soil substrate. Therefore, a test species which did not have the additive effect of cuticular uptake could provide indication of an upper limit of tolerance to a soil contaminant.

The soil-dwelling larval cranefly (Diptera: Tipulidae: *Leptotarsus* spp.) is proposed here as a novel test organism for the quantification of responses to soil metal contamination. This genus is easy to handle and also tolerates the laboratory environment, a key criteria for usefulness in this context (Ecobichon 1992). In a preliminary investigation (reported in Appendix C), *Leptotarsus* spp. was found to rapidly accumulate metals from a contaminated soil habitat. Analysis of the whole body metal concentrations by atomic absorption spectrophotometry (AAS) identified high levels of Cu and Zn had been accumulated by the larvae after a typical 28-day chronic exposure period.

This outcome prompted a further investigation, to examine the differential uptake and subsequent elimination of Cu and Zn by cranefly larvae, using survival and growth as parameters for measurement. I tested the hypothesis that the survival and growth of larvae would be dose-dependent and predicted a negative gradient of effect for both parameters. This is because the process of cellular detoxification (Simkiss and Taylor 1989, Hopkin 1990) was expected to

impose an energy cost on the larvae, which at incremental levels of concentration, would result in a reduction in the capacity to survive and grow (Storch 1984, Bengtsson *et al.* 1985, Maltby 1999). As detoxification was also expected to have a residual energy cost (Ma 1984, Maltby and Naylor 1990), I sought evidence for subsequent differentials in survival and growth following removal of the metal stressor. I predicted larval survival and growth would be highest in the control group which had not previously been exposed to metal spiked soils.

The outcomes of the 30 day toxicity test on larval craneflies identified (i) a LOEC (lowest observed effect concentration causing 20% or greater mortality) of 60 ppm Cu (pH 4.65) and a LOEC of 340 ppm Zn (pH 3.94); (ii) a NOEC (highest concentration causing 20% or less mortality) of 120 ppm Cu (pH 1.52) and a NOEC of 120 ppm Zn (pH 4.67); (iii) significantly impaired growth of larvae in soil treatments of 60 ppm Cu and 80 ppm Zn; (iv) the cranefly larvae were able to both rapidly sequester and rapidly expel ingested Cu and Zn. The experimental outcomes are discussed in relation to the bioavailability of metal and biotic pathways for sequestration and elimination.

B. MATERIALS AND METHODS

The experimental protocol was developed following a preliminary experiment. Details of this experiment are given in the Appendix C.

1. Larvae

During March 2001, approximately 2000 cranefly larvae were hand-sorted from the A horizon of a 21-year old mid-Canterbury pine plantation. The larvae were maintained for four weeks in a sieved mix of soil from this forest in 2 litre plastic containers in a controlled temperature cabinet at 12°C, 80% relative humidity and a 12 hour light/12 hour dark ratio.

Immediately prior to the commencement of the experiment, larvae were randomly allocated to 20 closed plastic petri dishes containing filter paper dampened with distilled water, to clear gut contents (Cain *et al.* 1995). Larvae were maintained in the petri dishes in the controlled temperature cabinet for 48 hours. The filter paper was changed twice daily. Gut clearance was assumed to be complete when exuviae no longer soiled the filter paper. Groups of 20 larvae were randomly selected, rapidly rinsed in distilled water and placed on clean filter paper to dry. Larvae were weighed and allocated to a treatment.

3. Whole body metal concentration (WBMC) of larvae

The concentrations of Cu and Zn in cranefly larvae were determined by atomic absorption spectrophotometry (AAS) at Bromley Wastewater Treatment Laboratory, Christchurch. The larvae were digested and analyzed using a modified version of EPA 3050B methodology. This method consists of digestion of the sample with elevated pressure (and therefore temperature) in special vessels made of teflon, conducted inside a purpose-built microwave oven. The sample is weighed out into the vessel and 4 ml nitric acid and 1 ml hydrogen peroxide are added to the vessel, which is then capped then processed. The samples are cooled, made up to volume and digestates analyzed directly using graphite furnace AAS for elements below 100 ppm concentration and direct flame AAS for concentrations greater than 100 ppm. Larvae from each soil treatment were examined as duplicate samples (between 0.1 and 0.2 gm dry weight samples) The WBMC of larvae for each treatment is given as the dry weight equivalent in parts per million (ppm) and is the average of the two duplicates.

4. Spiked soil treatments

Fresh soil (referred to as FORE) was removed from the 21-year old forest and stored prior to use in sealed plastic bags in a closed bin in a temperature-controlled room at 20°C. The lid of the bin was opened and soil aired regularly. Each experimental unit consisted of a plastic, 450 ml screw-top jar containing 300 gm dry weight equivalent of FORE soil. The lid of each container was perforated by a small central hole for aeration. The range of metal salt concentrations was selected based on USEPA benchmarks for earthworms and microbial heterotrophs (Will and Suter 1994), the limit levels for metals in agricultural soils in New Zealand (NZDH 1992) and the estimated background concentrations of Cu and Zn in the FORE soils (Anon. 1996)(see Appendix C).

Replicates were amended with either Cupric chloride ($CuCl_2$ $2H_20$, Univar, Ajax Chemicals, Sydney) or Zinc chloride ($ZnCl_2$ BDH Ltd., Poole, England) in solution (distilled water), calculated to bring the volumetric water content of the soil to 30%. Control treatments were unspiked FORE soils, and designated BCK (background levels of metals only). BCK treatments received a distilled water equivalent. For each metal tested, Cu and Zn, there were three treatments and 10 replicates within each treatment. Twenty control (BCK) replicates were used. The soil in each replicate was mixed thoroughly using a glass rod, then left to equilibrate in a controlled environment for 4 days prior to the addition of the 20 cranefly larvae to the soil surface.

4. Soil metal concentration

An accurate estimate of the concentration of metals in the spiked soils was established following AAS analysis. One 5 gm sample was taken from each replicate immediately prior to the addition of the larvae. The samples for each treatment were bulked, stored in sealed paper bags and oven dried at 105°C for 48 hours (McLaren and Cameron 1996). The concentrations of Cu and Zn were determined by the Christchurch Wastewater Treatment Plant using atomic absorption spectrophotometry (AAS) (Hopkin *et al.* 1989, Borovansky 1997). A standard method of nitric/perchloric acid digestion at 90°C for 2 hours was used, as this method dissolves all absorbed, chelated and otherwise bonded metals without attacking the crystalline metal content. Results are given as the mean of two duplicates. Metal concentrations are given as parts per million dry weight.

5. Soil pH

The pH of the soil (five randomly selected samples from each set of treatments) was measured 72 hours after spiking, after 30 days (at end of TREATMENT phase) and after 62 days (at the conclusion of the RECOVERY phase). Each 20 gm replicate of air-dry soil were placed in a beaker containing 50 ml distilled water (1: 2.5). The suspension was shaken by hand and the beaker then covered and left to stand overnight. A JENWAY 310 Microprocessor pH meter (Jenway Ltd., Essex) was standardized with buffer solutions of pH 4 and pH 7. The electrode was inserted into the suspension and pH was read to the nearest 0.01 unit. The electrode was rinsed in distilled water and excess water removed with tissue paper between readings. All measurements were made in a temperature-controlled room at 20°C.

6. Soil organic matter and carbon content

An indirect estimation of soil organic matter (SOM) and soil organic carbon (OrgC) was calculated from loss on ignition (LOI) values of FORE soils. There is good agreement between organic carbon content for Canterbury soils determined by chemical oxidation using the Walkley-Black procedure (Tiessen and Moir 1993) and by a direct combustion technique (Grewel *et al.* 1991). Five replicates of approx. 10 gm of air-dry soil were placed in porcelain crucibles and oven-dried at 105°C for 48 hours. Samples were cooled in a desiccator, weighed and then placed in a muffle furnace at 550°C for 4 hours. Samples were again cooled in a desiccator before re-weighing to calculate ash weight. Soil organic matter was estimated as the loss on ignition being the percentage weight loss that occurs when a soil is ignited in a furnace. The assumption that SOM contains 58% carbon was used to estimate soil organic carbon content

7. Experimental protocol

7.1 Treatment Phase

Replicates were maintained for 30 days (12.5°C, 80% relative humidity, 12/12hr-light/dark period) in a controlled temperature cabinet. Containers were repositioned twice weekly within the cabinet to minimize abiotic variation. After 30 days, all larvae from each replicate and treatment were rinsed briefly in distilled water to remove extraneous soil particles, blotted dry, then placed on filter paper in closed petri dishes in the same replicate groups and returned to the controlled temperature cabinet for 48 hours to evacuate gut contents. The filter paper was changed twice daily. Following gut clearance, all active larvae from each replicate were weighed. Specimens observed to be moribund were treated as mortalities.

For each replicate, 50% of all surviving larvae were randomly selected and immediately killed in boiling water, cooled to room temperature, then transferred to glass vials for fixation in 8% formaldehyde for 24 hours. Larvae were washed through two changes of 70% alcohol and stored in glass vials for later histological or AAS analysis. For each soil treatment, at least 30 larvae were bulked to form one sample and processed in duplicates (refer to Appendix C). The larval WBMC for each metal was given as the average of the duplicates.

7.2 Recovery Phase

The remaining 50% of surviving larvae from each replicate were returned to clean 450 ml screw-top jars containing 300 gm dry weight equivalent of unspiked FORE soil. Distilled water had previously been used to adjust the volumetric moisture content to 30% and containers left to stabilize for 4 days under conditions previously described in the controlled environment cabinet. Replicates were placed in the controlled temperature cabinet for a further 30 days, after which the process of gut clearance, weighing of survivors and retention for later AAS or histological analysis was repeated.

8. Analysis

The mean number of larvae surviving in each soil treatment at the conclusion of each experimental phase was calculated and analyzed (ANOVA, SAS Institute V8). Data are presented graphically as the total number of larvae surviving in each treatment and each phase. Larval growth was calculated as the mean change in oven dry biomass within replicates and treatments between the start and end of each experimental phase (Two way ANOVA Proc GLM, SAS Institute V8). Data are presented graphically as the mean (±SD) biomass of larvae at the start and end of each experimental phase.

Homogeneity of variance between treatment means was examined prior to analysis to avoid violation of ANOVA assumptions. As a difference between treatment means was predicted from the hypotheses, Duncan's Post Test was used to identify significant pairings.

Accumulation factors (A_f) for Cu and Zn were calculated by comparing the element concentration in the larvae with their substrate. An $A_f > 1.0$ is indicative of element enrichment in invertebrates, whilst an $A_f < 1.0$ suggests the discrimination of elements from one trophic level to the next (Roth 1992).

The NOEC and LOEC for both Cu and Zn in relation to survival and growth were calculated where appropriate. The NOEC (no observed effect concentration) is defined as the highest applied concentration of the chemical of interest causing a reduction of 20% or less in a measured response. The LOEC (lowest observed effect concentration) is defined as the lowest applied concentration of the chemical of interest causing a reduction of 20% or greater in a measured response (Will and Suter 1994).

C. RESULTS

1. The concentrations of Cu and Zn in spiked FORE soils

The soil metal concentrations for each treatment following AAS analysis are given in Table 6.1. Values are expressed to the nearest 10 ppm. BCK refers to the background (unspiked) concentration of the stated metal occurring naturally in the FORE soil.

2. Soil pH and organic carbon

The pH of BCK soil was stable throughout the experimental period (Table 6.1). Spiking of the FORE soil with metal salts increased soil acidity. A decrease in soil pH was recorded for all soil treatments after 72 hours. At the end of the TREATMENT phase, a substantial and dramatic fall in pH was recorded for both the 120 and 150 ppm Cu treatment groups. Previous analysis of the FORE soil had identified the SOM to be 12.09% and the Org.C to be 7.01%.

Table 6.1 The pH of soil treatments prior to spiking, after 72 hours, after 30 days and at the conclusion of the experiment (62 days). BCK = Background control group.

SOIL TREATMENT GROUP	After 72 HOURS pH	END TREATMENT PHASE (after 30 days) pH	END RECOVERY PHASE (total 62 days) pH
BCK	5.10	5.11	5.10
Cu (ppm)			
60	4.66	4.65	
120	4.51	1.52	
150	4.50	1.46	
Zn (ppm)			
80	4.84	4.83	
120	4.73	4.67	
340	3.97	3.94	

3. WBMC in larvae: TREATMENT and RECOVERY phases

The outcomes of the AAS analysis of the WBMC in larvae are summarized in Table 6.2. In untreated control soils (BCK), larvae have a higher WBMC of both Cu and Zn than that of the soil. During the TREATMENT phase, the WBMC of all larvae increased with incremental soil metal concentrations. There was a decrease in the WBMC of larvae following the subsequent transfer of larvae to, and maintenance in, control soil (RECOVERY phase). There was a good indication that the WBMC of Cu and Zn may fluctuate normally, as shown by the A_f and percentage change values for larvae in the control groups.

4. Accumulation Factors (A_f): TREATMENT and RECOVERY phases

Positive metal enrichment ($A_f > 1$) of larvae occurred during the TREATMENT phase for all metal concentrations tested (Table 6.2). Although the WBMC was found to increase with incremental soil metal concentrations, it is likely that the accumulation of Cu and Zn is non-linear. This possibility was seen at the conclusion of the TREATMENT phase, where the A_f appeared to reach an asymptote at 120 ppm Cu and 120 ppm Zn. At the conclusion of the RECOVERY phase, the WBMC of larvae from both Cu and Zn treatments was found to have decreased. For larvae in the Cu experimental groups, the A_f values calculated for the RECOVERY phase were higher than for the TREATMENT phase. This indicated that the Cu WBMC had not returned to a baseline value. For larvae in the Zn experimental groups, the A_f values calculated for the RECOVERY phase had not substantially changed from those at the end of the TREATMENT phase.

Table 6.2 Background (BCK) and AAS estimates of the concentration of metal in the soil for each experimental group, the whole body metal concentrations (WBMC) of cranefly larvae after 30 days (TREATMENT phase) and after the RECOVERY phase. Accumulation factors (A_f) for each metal were calculated from the relationship between the WBMC and the concentration of that metal in the soil substrate (note RECOVERY phase A_f calculated from BCK soil concentration for Cu or Zn respectively). WMBC values derived from the digestion of at least 30 larvae randomly selected from 10 replicates from each treatment and bulked for digestion. Samples were processed in duplicate and given as the average of the two values.

*Insufficient larvae for analysis.

EXPERIMENTAL GROUP	TREATMENT PHASE WBMC (ppm)	A_f	RECOVERY PHASE WBMC (ppm)	A_f
Copper (ppm)				
BCK (5)	14.3	2.9	12	2.4
60	200	3.3	32	6.4
120	625	5.2	78	15.6
150	725	4.8	95	19
Zinc (ppm)				
BCK (50)	120	2.4	106	2.1
80	165	2.1	118	2.4
120	644	5.4	230	4.6
340	1049	3.1	*	*

5. Survival of larvae: TREATMENT and RECOVERY phases

The survival of larvae during the TREATMENT phase was significantly affected by the concentration of metal in the soil ($F = 27.36$, $P < 0.0001$) (Table 6.3, Figure 6.1).

Table 6.3 ANOVA summary statistics for the effect of soil metal concentration on larval survival in the TREATMENT phase.

Source	df	Sum of Squares	Mean Square	F value	P
MODEL	6	1313.69	218.95	27.35	< 0.0001
ERROR	73	584.3	8		
CORRECTED TOTAL	79	1897.99			

In the TREATMENT phase, no evidence was found for a dose-dependent negative gradient of effect for either Cu or Zn soil treatment groups (Table 6.4). Larval survival in the 340 ppm Zn spiked soils was significantly reduced compared with larvae from all other Zn treatments. The high mortality in the 340 ppm Zn treatment resulted in an insufficient number of larvae from that group being available to take forward to the next phase. Significantly fewer larvae survived in the 60 ppm Cu treatment group than in all other Cu soil treatments.

Table 6.4 Significantly different pairings identified by Duncan's Test between spiked soil treatments for the survival of larvae during the TREATMENT phase. Means with the same letter are not significantly different at $P < 0.05$.

Duncan Grouping			Mean	N	Treatment Group
	A		19.1	10	120 ppm Zn
	A		18.7	10	80 ppm Zn
B	A		18.1	20	BCK
B	A	C	17.8	10	120 ppm Cu
B	A	C	15.9	10	150 ppm Cu
		C	15.3	10	60 ppm Cu
	D		5.9	10	340 ppm Zn

The transfer of all larvae in their soil-treatment groups to an unspiked soil equivalent for the RECOVERY phase had a weakly significant effect on larval survival ($F = 2.98$, $P = 0.0176$) (Table 6.5, Figure 6.1). Duncan's Post Test was used to identify significant differences in survival between treatment means for this phase (Table 6.6). Larvae which had not previously been exposed to spiked soils had a higher rate of survival than larvae from all Cu treatments and whilst a dose-dependent effect for Cu was found, survival was only significantly affected in the 150 ppm Cu group ($P < 0.05$).

Table 6.5 ANOVA Summary statistics for the effect of soil metal concentration on larval survival in the RECOVERY phase.

Source	df	Sum of Squares	Mean Square	F value	P
MODEL	5	104.69	20.94	2.98	0.0176
ERROR	63	442.12	7.01		
CORRECTED TOTAL	68	546.81			

Table 6.6 Significantly different pairings between treatments for the mean survival of larvae in RECOVERY phase, identified by Duncan's Test. Note each group of replicates were maintained in BCK soil during the RECOVERY phase. Means with the same letter are not significantly different at $P < 0.05$.

Duncan Grouping		Mean	N	Treatment Group
	A	6.8	20	BCK
	A	6.4	9	60 ppm Cu
	A	5.5	10	120 ppm Zn
B	A	5.3	10	120 ppm Cu
B	A	5.3	10	80 ppm Zn
B		3	10	150 ppm Cu

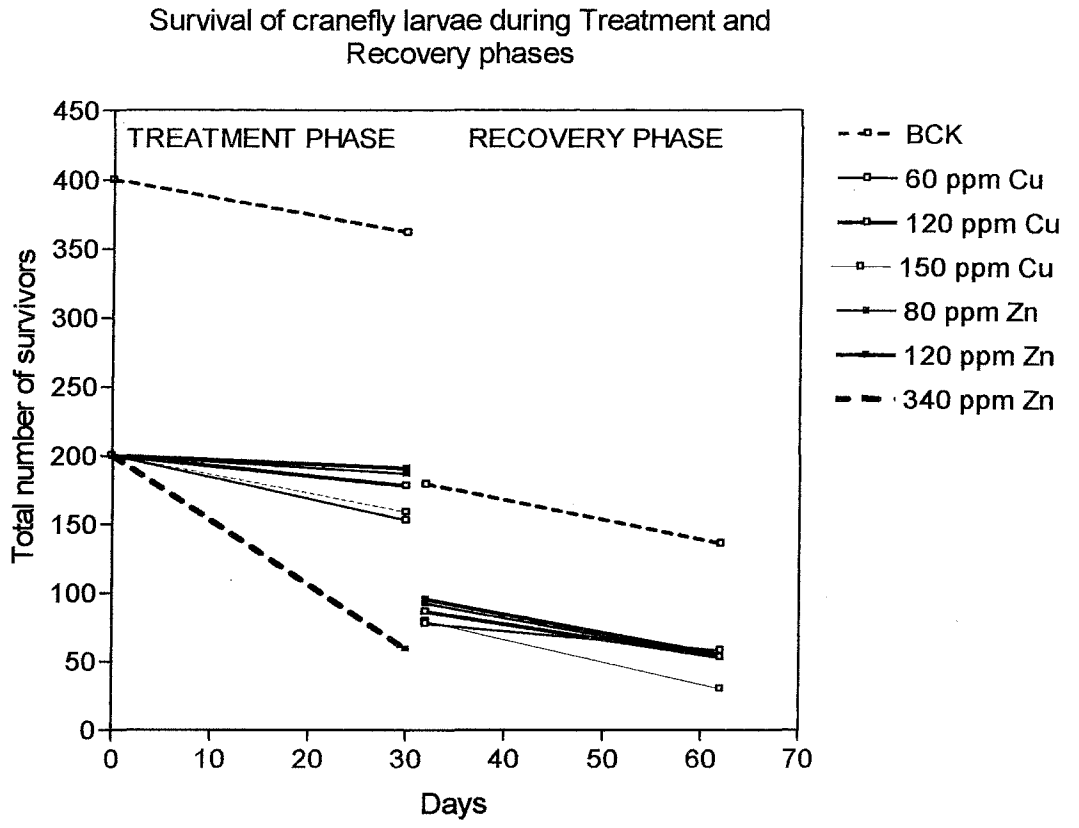

Figure 6.1 Differential survival of larvae from soil treatment groups and between the two experimental phases, TREATMENT and RECOVERY. Values are the total of all larvae surviving from each group of replicates, within each of the metal concentrations, at the conclusion of each phase. Spiked soil concentrations tested were Control (BCK), 60 ppm Cu, 120 ppm Cu, 150 ppm Cu, 80 ppm Zn, 120 ppm Zn, 340 ppm Zn. The gap between 30-32 days is the period of gut clearance between experimental phases.

At the conclusion of the TREATMENT phase, the NOEC for larval survival in Cu spiked soils was 120 ppm Cu (11% mortality). Similarly, the LOEC was calculated to be 60 ppm Cu (23.5% mortality). In Zn spiked soils, the NOEC for larval survival was 120 ppm Zn (4.5 % mortality) and the LOEC was calculated to be 340 ppm Zn (70.5% mortality).

6. Growth of larvae: TREATMENT and RECOVERY phases

Larval growth was significantly influenced by the concentration of metal in the soil (Two way ANOVA, $F = 4.46$, $P < 0.001$) (Table 6.7). The time of biomass measurement within a phase (start or end) was also highly significant ($F = 103.64$, $P < 0.0001$). This indicated that a change in biomass occurred between the start and end of the experimental phase. This change was positive for all treatment groups. There was also a significant interaction between metal concentration and time and it was concluded that there were differences between the measured biomass within treatment groups at the start and at the end of the experimental phase ($F = 4.80$, $P < 0.001$).

There was no evidence of a negative gradient of effect of incremental soil metal concentration on larval growth, for either Cu or Zn soil treatment groups. However, larval biomass was significantly lower in all tested Cu concentrations than in the BCK group (Table 6.8). It is noted here also that larval biomass in the 80 ppm Zn treatment group was lower than in the BCK group, although no effective difference was found for the larvae in the 120 ppm Zn group. As biomass gains were found for larvae in all treatment groups during this phase, calculation of the NOEC and LOEC values was not appropriate.

Although the 340 ppm Zn treatment group was excluded from the analysis at this point, results are included graphically for completeness. Separate graphs for larval biomass for the two metals tested during both experimental phases are shown in Figure 6.2 (Cu) and Figure 6.3 (Zn).

Table 6.7 Summary statistics for two-way ANOVA for larval biomass. Comparisons were drawn between larval biomass in treatment groups at the start and the end of the TREATMENT phase.

Source	df	Sum of squares	Mean Square	F value	P
METAL	5	0.00996	0.000199	4.46	0.0009
TIME	1	0.00463	0.004632	103.64	< 0.0001
METAL*TIME	5	0.00107	0.00021477	4.80	0.0005

Table 6.8 Significantly different pairings identified by Duncan's Test for larval biomass in the TREATMENT phase. ($P < 0.05$). Means with the same letter are not significantly different at $P < 0.05$.

Duncan Grouping		Mean	N	Treatment Group
	A	0.02799	40	BCK
B	A	0.02740	19	120 ppm Zn
B	C	0.02348	20	80 ppm Zn
	C	0.02314	20	120 ppm Cu
	C	0.02287	20	60 ppm Cu
	C	0.02094	20	150 ppm Cu

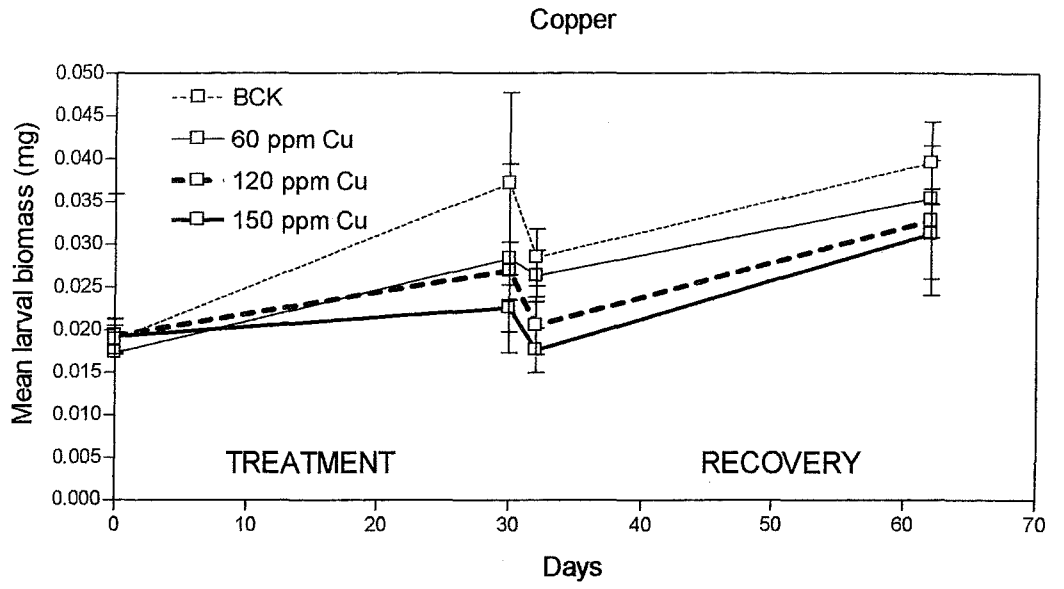

Figure 6.2. Mean (±SD) larval biomass (mg) after 30 days in Cu spiked soil treatments (TREATMENT phase) and after 30 days in unspiked soils (RECOVERY phase).

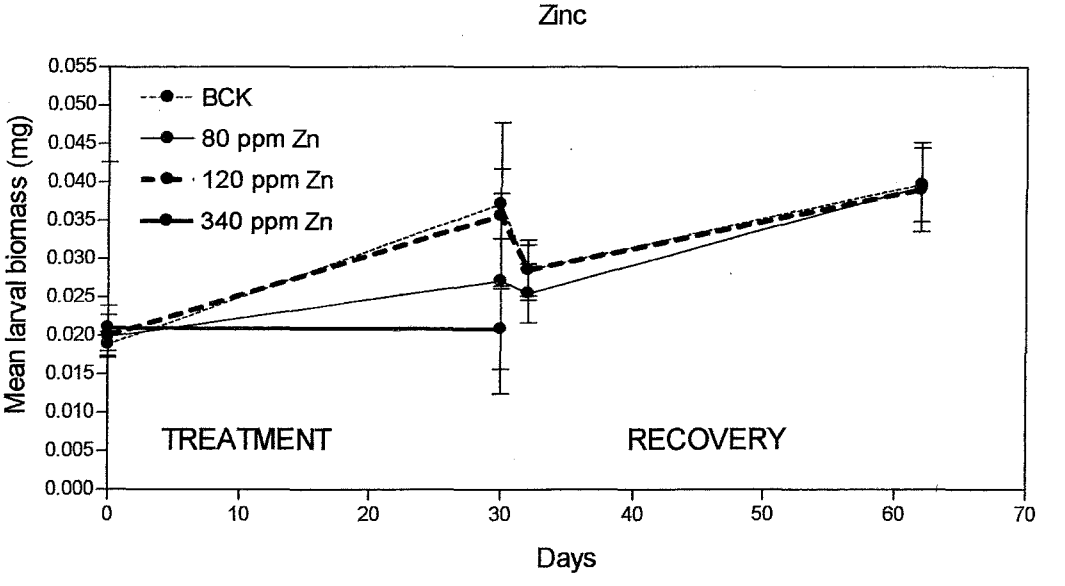

Figure 6.3. Mean (±SD) larval biomass (mg) after 30 days in Zn spiked soil treatments (TREATMENT phase) and after 30 days in unspiked soils (RECOVERY phase).

Previous exposure to contaminated soil had a significant effect on the growth of larvae (Two way ANOVA, $F = 15.91$, $P < 0.0001$) (Table 6.9). The time of biomass measurement was a significant main effect, indicating larvae grew during this phase ($F = 207.42$, $P < 0.0001$). The interaction between metal group and time was not significant.

Table 6.9 Summary statistics for two-way ANOVA for larval biomass. Comparisons were drawn from the mean larval biomass in treatment groups at the start and the end of the RECOVERY phase.

Source	df	SS	Mean Square	F value	P
METAL	5	0.001772	0.0003544	15.91	< 0.0001
TIME	1	0.004619	0.004619	207.42	< 0.0001
METAL*TIME	5	0.000044	0.000009	0.4	0.8453

Larvae, which had not previously been exposed to spiked soils gained more biomass than did larvae from all Cu treatments. There was evidence of a significant and negative gradient of effect for the Cu treatment groups ($P < 0.05$) (Table 6.10). Previous exposure to Zn had no effect on larval growth during this phase. At the conclusion of the RECOVERY phase, larval growth was at least equivalent to or greater than at the conclusion of the TREATMENT phase. As biomass gains were found for larvae in all treatment groups during this phase, the calculation of NOEC and LOEC values was not relevant.

Table 6.10 Significantly different pairings identified by Duncan's Test for larval biomass in the RECOVERY phase. ($P < 0.05$). Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	Treatment Group
	A	0.03404	40	BCK
B	A	0.03327	19	120 ppm Zn
B	A	0.03242	20	80 ppm Zn
B		0.03088	18	60 ppm Cu
	C	0.02670	20	120 ppm Cu
	C	0.02411	19	150 ppm Cu

D. DISCUSSION

Spiked soils increase soil pH

Spiking of the soil with Cu and Zn metal salts resulted in a progressive increase in the acidity of the soils. It is clear that at least the 120 ppm Cu and 150 ppm Cu treatments had not equilibrated prior to the placement of larvae in the test substrate. Given that standard soil conditions in toxicological experiments generally assume a pH of 6 (van Straalen and Bergema 1995) the experimental conditions imposed on the larvae were extremely acidic. This is likely to have increased the bioavailability of the metals in the soil pore water and the quantity of metal ingested, either directly or indirectly (Crommentuijn *et al.* 1997, Rieuwerts *et al.* 1998).

The acid soils may have restricted activities carried out by microbial heterotrophs (Bardgett *et al.* 1994) and limited fungal growth. This in turn, may have restricted the availability of food resources for the cranefly larvae. Although the larvae are (in the main) fungal feeders, the specific soil fungi present and their disposition to bioaccumulate metals is not known (Lodenius 1981,

Sayer *et al.* 1999). Delimitation of the heterotrophic response could help in understanding bioaccumulation factors and tolerance to metal contaminants (Bengtsson *et al.* 1985, Colpaert *et al.* 2000). Subsequent studies may benefit by integrating microbial responses with that of the invertebrate test organism.

The highly acid conditions may have influenced the bioavailability of the metals to larvae, through induced changes in speciation and solubility in the soil. Metals form organo-metal complexes and the rate of formation is directly related to the amount of organic carbon in the soil (Gao *et al.* 1997). Previous studies have shown the percentage organic carbon in soil to be a strong determinant of Cu bioavailability and toxicity to lumbricid earthworms (Streit and Jaggy 1983, Ma 1984). However, as the percentage organic carbon in the experimental soils was generally comparable to that of substrates used in other studies (Will and Suter 1994)it is unlikely that complex formation acted to reduce bioavailability.

The experimental procedure may have been enhanced had appropriate buffers been used to regulate soil acidity. However, by not using a buffer to reduce acidification, extreme conditions serve to highlight physiological responses and provide confidence in the determination of guideline limits. Further experimentation implementing buffers or using pH as a variable is recommended to better understand the larval responses.

Whole body metal concentration (WBMC) in larvae

Larvae were found to actively accumulate both Cu and Zn during the TREATMENT phase. They subsequently eliminated a substantial proportion of the accumulated metals when returned to and maintained in control soils. Although the WBMC was found to increase with incremental soil metal concentrations, there is some evidence that accumulation of Cu and Zn may be non-linear. This possibility was seen at the conclusion of the TREATMENT phase, where the A_f appeared to reach an asymptote at 120 ppm Cu and 120 ppm Zn. This interpretation remains inconclusive without further testing at relevant soil metal concentrations. For larvae in the Cu experimental groups, the A_f values calculated for the RECOVERY phase were higher than for the TREATMENT phase, indicating the Cu WBMC had not returned to a baseline value. For larvae in the Zn experimental groups, the A_f values calculated for the RECOVERY phase were not greatly changed. These observed differences are likely to reflect the risk of retaining the metals, such that excess Cu may present a greater risk than excess Zn. Elimination is understood to have an energy cost. Histological examination of larvae is expected to identify structural differences, which could provide support for the energy allocation theory.

The cause of WBMC fluctuations observed in the control (BCK) larvae during the experiment may be a reflection of periodic elimination of detoxified material such that equilibrium is reached. The regulation of the WMBC may be influenced by a variety of factors, including behaviour, physiology, feeding activity, bioaccumulation factors in preferred food resources, non-selective ingestion of organ-metal complexes, grazing of organic detritus coated with dissolved metal salts, elimination of sequestered metals in exuviate or shedding of metal burdens lodged in the exoskeleton between instars (Siepel 1995, Gräff *et al.* 1997, Grelle *et al.* 2000). Although there was good agreement between AAS duplicates, the low level of replication for the AAS analysis suggests results should be viewed with caution.

The background WBMC for wild populations of tipulid larvae (as shown in this study) are relatively low compared with published values. For example, *Tipula scripta* larvae taken from an uncontaminated 80-year-old Norway spruce (*Picea abies* L. Karsten) monoculture had a WBMC of 221 ppm Cu (±1.6) and 115 ppm Zn (±9); *Tipula* spp. Type B had a WBMC of 26 ppm Cu (±2.6) and 149 ppm Zn (±21) (Roth 1992). These concentrations were against mean background levels in the respective humus layers (O_L, O_F, and O_H combined) of 13.3 ppm Cu (±5.24) and 68 ppm Zn (±15.64). The differences in accumulation factors were attributed to selective grazing behaviour, seasonal variation in the concentration of soluble metal in the soil pore water, as well as varying tolerance and elimination capacities of species. It is this variability that makes the strict delimitation of metal sensitivities such a difficult task. It also highlights the need to expand the number of species used to set limits for acceptable levels of soil contamination.

Survival of larvae during TREATMENT and RECOVERY phases

The absence of a clear dose-dependent effect for either Cu or Zn on larval survival during the TREATMENT phase indicates that with the exception of 340 ppm Zn, the concentrations tested were within the biologically tolerable capacity of the larvae,

The isolated and significant difference in survival of larvae in the 60 ppm Cu group during the TREATMENT phase was difficult to explain. It was unlikely to be due to acidity alone, as larvae in higher Cu concentrations in soils where the pH was at least 0.15 units more acidic did not suffer a significant mortality effect. One possible explanation for apparently aberrant outcomes in toxicity tests may be related to the suppression or stimulus of gut fauna, including parasites sensitive to specific metals (Bengtsson *et al.* 1985). This could be a valuable area for further investigation.

The reduction in survival during the RECOVERY phase, which was experienced by all groups could be attributed to the stress of the experimental protocol, including, for example, the process of gut clearance. For all groups, the survival rate was equivalent to, or lower, than that experienced during the TREATMENT phase. Evidence of a negative dose dependent effect on larvae previously exposed to Cu amended soils appeared dose-dependent and significant for the 150 ppm Cu group. There are limitations in explaining possible causes and effects using survival data alone, especially where a chronic rather than toxic response is anticipated. Although survival is regularly used to assess the toxicity of a soil contaminant, it has been noted to be a less sensitive measure than reproduction or growth

Growth of larvae during TREATMENT and RECOVERY phases

Larvae in all experimental groups exhibited a positive growth increment during both phases. At the conclusion of the RECOVERY phase, all larvae had gained biomass, which was at least equivalent or greater than that recorded at the end of the TREATMENT phase. However, differential growth responses, some of which were significantly lower than the control group, and were clearly not dose-dependent, were found for both experimental phases. For example, growth in the 80 ppm Zn treatment was impaired but interestingly, growth in the 120 ppm Zn treatment was not.

The growth of larvae in all Cu amended soils was significantly lower than untreated controls. Larvae may have been allocating a substantial amount of energy to sequestration and detoxification, which is not differentiated easily by simple biomass measurement. Thus, the chronic response to uptake of the essential metal Cu may be energy related. Allocation of reserves to detoxification may be better discerned at the tissue level, seen, for example, as a reduction in the number and size of fat bodies, as well as the location of metal sequestration. This point is examined in a subsequent chapter.

Impaired larval growth may have also been due to a reduction in feeding activity, which may be a reflection of reduced availability of food resources, palatability or suppression of the feeding reflex by some unknown factor. These possibilities warrant further study.

Cranefly larvae as response indicators in chronic toxicity tests: key outcomes and comparisons with other indicator species

The direct comparison of test outcomes with published response by other species is confounded by the wide variety of test substrates, organic carbon content of the substrate, metal species and

durations of exposure. However, this variability can be advantageous because it encompasses a wide range of possibilities from which baselines might be drawn with varying levels of confidence. A summary of key findings is provided in Table 6. 11.

Table 6.11 Summary of chronic toxicity test outcomes for a variety of test species. OECD = commercial artificial soil

Test Organism	Substrate	Metal (ppm)	Response	Reference
L. rubellus	$CuSo_4$ to loamy sand field soil with 5.7% organic matter, pH 4.8	148 ppm Cu	26% decrease in production	(Ma 1984)
L. rubellus	As above, topped with leaf litter,	131 ppm Cu (54 ppm Cu no effect)	42% decrease in cocoon production	Ma 1984
E. fetida	copper acetate to horse manure	500 ppm Cu	24% decrease in cocoon production	Malecki *et al.* 1982
E. fetida	copper acetate to horse manure	300 ppm Cu	no effect on adult growth	Malecki *et al.* 1982
Octolasium cyaneum	Cu ($CuSO_4$) to a Brown soil (3.2% organic carbon)	180 ppm Cu	14-day LC_{50} (mortality)	Streit and Jaggy 1983
Octolasium cyaneum	Cu ($CuSO_4$) to a Rendzina soil (14% organic carbon)	850 ppm Cu	14-day LC_{50} (mortality)	Streit and Jaggy 1983
L. rubellus	sandy loam soil using $CuCl_2$ (pH 7.3, 8% organic carbon)	1000 ppm Cu (no effect at 150 ppm)	82% decrease in survival	Ma 1982
soil micro-arthropods	acid sandy forest Cu (as Cu SO_4)	400 ppm	abundance fell by approx 50% after 7 days	Parmelee *et al.* 1993
Caenorhabditis elegans	Cu (added as $CuCl_2$) to a silt loam soil with a high percentage of organic matter	400 ppm	LC_{50} after 24 hrs (mortality)	Donkin and Dusenbery 1993
E. fetida	$CuCl_2$ mixed with OECD soil	100 ppm Cu (no effect at 32 ppm)	32% reduction in growth	van Gestel *et al.* 1991
E. andrei	21 days in OECD soil (pH 6)	180 ppm Cu (no effect at 120 ppm)	36% decrease in cocoon production	van Getsel *et al.* 1989
E. fetida	soluble Cu added to OECD soil	1000 ppm Cu	growth unaffected	Neuhauser et al 1984
E. fetida	OECD soil, pH 6.3	555 ppm Zn	LC_{50} (survival)	Spurgeon *et al.* 1994
E. fetida	OECD soil, pH 6.3	276 ppm Zn	LC_{50} (cocoon production /growth)	Spurgeon *et al.* 1994

The key outcomes of the study using *Leptotarsus* spp. are as follows:

(i) A 23.5% reduction in survival at 60 ppm Cu (stony silt loam, pH 4.65, 7.01% organic carbon) after 30 days when the WBMC was 200 ppm Cu.

(ii) Wild *Leptotarsus* spp. populations in pine forests found to have a WBMC of 14.3 ppm Cu.

(iii) A 70.5% reduction in survival at 340 ppm Zn (stony silt loam, pH 3.94, 7.01% organic carbon) after 30 days when the WBMC was 1049 ppm Zn.

(iv) Wild *Leptotarsus* spp. populations in pine forests found to have a WBMC of 120 ppm Zn.

(v) Growth significantly impaired at 60 ppm Cu after 30 days (pH 4.65), when the WBMC was 200 ppm Cu.

(vi) No difference in growth impairment at higher tested concentrations of Cu after 30 days in substantially more acidic soils and up to three times higher WBMC of Cu than noted in (v)

(vii) Growth significantly impaired at 80 ppm Zn after 30 days (pH 4.83) when the WBMC was 156 ppm Zn.

(viii) Growth not impaired at 120 ppm Zn after 30 days (pH 4.73) when the WBMC of Zn was almost four times higher than noted in (vii)

Comparison with Table 6.1 suggests that the larval cranefly *Leptotarsus* spp. response is at least as sensitive to metal contamination as earthworms. Their use as an environmental bioindicator is therefore recommended.

The energy budget of invertebrates in metal-contaminated habitats

There was good evidence that impaired growth during exposure to metal-contaminated substrates affects the energy budget of *Leptotarsus* spp. Metal detoxification strategies potentially involve the loss of scope for growth as shown by Maltby and Naylor (1990). This is expected to result in altered body chemistry or changes to cellular physiology, with subsequent effects on other biological processes (Filshie *et al.* 1971, Doughtie and Ranga Rao 1974). For example, a reduction in glycogen and lipid concentrations in the haemolymph of *Lymantria dispar* (Lepidoptera) larvae exposed to Zn have been demonstrated (Bischof 1995).

Conclusions

This experiment identified the susceptibility of *Leptotarsus* spp. to a soil substrate artificially contaminated with Cu or Zn at levels expected to elicit a chronic response. *Leptotarsus* spp. is a

sensitive indicator of metal contamination and appears to have a chronic response which is at least equivalent to that of more commonly used earthworms. The lack of a gradient of response to the tested concentrations presents a problem in interpreting the outcomes. However, the study confirms that the relative success of an organism in a contaminated environment may depend on its capacity to sequester, detoxify and eliminate ingested metals excessive to dietary requirements. This activity clearly requires an energy allocation. Evidence for differential effects on the energy reserves at the tissue level on cranefly larvae are explored in Chapter Seven.

CHAPTER SEVEN

THE CYTOTOXIC EFFECT OF CU AND ZN ON THE LARVAL CRANEFLY *LEPTOTARSUS* (DIPTERA:TIPULIDAE)

A. INTRODUCTION

The physiological responses of soil invertebrates to an environment contaminated with heavy metals have often been observed as diverse ultrastructural alterations at tissue, cellular and sub-cellular levels (Filshie *et al.* 1971, Humbert 1979, Storch 1984, Pawert *et al.* 1996). The level of effect has been shown to be dependent upon the metal species as well as the relative sensitivity of the cells and organelles (Kohler and Triebskorn 1998). Whilst many invertebrates are known to actively sequester and detoxify surplus ingested metals, these processes may impose an energy cost which could otherwise be used for bodily processes, such as growth (Neuhauser *et al.* 1984, Maltby and Naylor 1990, Donker 1992, Reinecke and Reinecke 1996, Spurgeon and Hopkin 1996).

The growth of invertebrates in a metal-contaminated environment may be limited by a combination of direct and indirect, physical and chemical effects. For example, the sequestration of the bio-inactive metal fraction has been shown to direct energy into enhanced metallothionien synthesis, whilst the bio-available fraction may cause the competitive inhibition of a multitude of cellular processes (Dallinger 1994, Morgan *et al.* 1999). As a result, defects and disorganization in cellular architecture may impair nutrient uptake and processing (Lee *et al.* 1993, Pawert *et al.* 1996). Cellular repair may place an additional and indirect tax on energy reserves.

The diversion of energy reserves into metal detoxification varies according to the ecology and physiology of the species in question. For example, the isopod *Porcellio scaber* utilizes a storage detoxification system, whilst the spider *Dysdera crocata* voids stored metals at the end of each digestive cycle (Hopkin 1990). As a consequence, the whole body metal concentration of *P. scaber* increases during a lifetime, whilst that of *D. crocata* does not deviate from normal over the long term. The adult housefly *Musca domestica* employs storage detoxification, by which metal-containing concretions in the midgut epithelium are cumulative with age (Sohal *et al.* 1977).

Copper and zinc are two metals which have been extensively studied in relation to their toxicity to invertebrates and the detoxification pathways employed (Ma 1984, Maltby and Naylor 1990, Hopkin and Hames 1994, Spurgeon *et al.* 1994, Posthuma *et al.* 1997). Both metals are essential and are required by invertebrates for cell differentiation, growth and enzyme regulation Pathways for their uptake exist at the cellular level. However, an efficient excretory mechanism is necessary for copper because it is also toxic. Most previous studies investigating copper and zinc toxicity have used earthworms, gastropods, isopods and collembolans. The use of larval dipterans is not widespread, despite their high level of ecological relevance, abundance and amenability to laboratory conditions (Frouz 1999).

It is likely that larval dipterans probably employ a storage detoxification system in which surplus zinc ions are bound intracellularly to phosphate-rich granules (Type A pathway) or surplus copper ions to sulphur-rich granules (Type B pathway) (Hopkin 1990). Following intracellular precipitation, there is no evidence that these granules can be dissolved and can only be excreted if cell contents are voided into the lumen of the digestive tract. Concretions and granular formations containing copper and zinc have been observed in the cuprophilic cells of the midgut of adult *Musca domestica* (Diptera: Muscidae) (Sohal *et al.* 1977) and larval *Drophila* sp. (Diptera: Drosophilidae) (Filshie *et al.* 1971, Tapp and Hockaday 1977). Copper is known to be absorbed by *Lucilia* sp.. larvae (Diptera: Calliphoridae) in the midgut where the pH is between 3.3 and 3.6 (Waterhouse and Day 1953). The midgut tissues are therefore likely sites at which cellular ultrastructure may be affected by chronic metal contamination.

Soils and the soil biota suffer from chronic metal contamination in locations used to dispose of metal-contaminated material, such as biosolids (Cameron *et al.* 1997). The accumulation of metals in soils is a limiting condition on the sustainability of a disposal or redistribution programme (Cameron *et al.* 1994, Anon. 1998a). However, the establishment of guidelines protecting the biota and ecological processes, is reliant on understanding the resilience, resistance and uptake parameters of the widest range of ecologically relevant species. For this reason, the larval cranefly *Leptotarsus* spp. is explored as a possible tool for future toxicological testing.

In the previous chapter, I demonstrated significant constraints on the growth of cranefly larvae *Leptotarsus* spp. during a 30 day laboratory trial in copper or zinc salt spiked soils, compared with the control state. In this chapter, a semi-quantitative methodology was used to identify the possible histological basis for the growth response, using a light microscope to examine the resorptive tissues of larvae using stains specific for either copper or zinc to localize sequestered

material and reactive ions the tissues. Both fixation and ultrastructural differences were noted between treatment groups. These outcomes are discussed in relation to the histological processes employed and possible linkages with the impaired growth observed.

B. MATERIALS AND METHODS

Gut-cleared larvae (Cain *et al.* 1995) were killed in boiling water and fixed for 24 hours in 8% formaldehyde, washed through two changes of 70% alcohol and stored at that concentration prior to dehydration. Processing and imaging was carried out in association with technical staff from CSIRO Entomology and The Australian National University (Canberra).

Larvae were sequentially dehydrated to 100% ethanol overnight in a Tissuetek Vacuum Infiltration Processor 1000 (1986 Sakura, Japan) with pressure and vacuum at 40°C. Samples were cleared in chloroform for 45 minutes at 40°C under pressure and vacuum. Chloroform was preferred to xylol as it is gentler and does not harden the tissues (Anne Prins, pers. comm.). Specimens were transferred through three, 30-minute changes of wax (Paraplast) at 60°C under pressure and vacuum. Each block made represented four larvae from the control group and four from each of the 60, 120 and 150 ppm Cu and 80, 120 and 340 ppm Zn treatment groups (see Chapter Six).

Longitudinal sections (4μm thick) were cut using a Leitz 1512 microtome (Feather S35 blades) and mounted on superfrost slides. Slides were heat fixed for 1 hour at 60°C and dewaxed in two, 2-minute changes of xylol, followed by two changes of absolute alcohol (10 dips each change). One change each of 90 and 70% alcohol was followed by at least two minutes wash in tap water.

Gill's No. 3 Haematoxylin was used to stain the slides for 3 minutes. Sections were then washed well in tap water, dipped into ammonia and water (0.2ml in 200 ml tapwater) and given another good wash. Sections were counterstained with eosin (1% eosin in 80% alcohol) for 2 minutes after which they were run through four changes of absolute alcohol and two changes of xylol. Coverslips were placed over sections and sealed with DPX glue (a solvent-based mountant). The two adjacent longitudinal sections from each block were dewaxed as described above and stained to identify tissue-level accumulation sites of Cu or Zn. A rubeanic acid stain was used for copper and an alkaline diphenylthiocarbazone (dithizone) stain for zinc (Prosi and Dallinger 1988, Kiernan 1990). Rubeanic acid was expected to give a deep greenish-black colour reaction with copper salts and dithizone a blue-black response (Pearse 1972).

Slides were viewed with a semi-automated computerized image analysis system consisting of a light microscope (Leitz Diaplan) connected to a high-resolution digital camera (Zeiss ProgRes 3012). Images were processed using Photoshop software.

1. Analysis

The reported outcomes are based upon microscopic examination of stained longitudinal sections of four replicate larvae for each metal treatment group plus control. Sections were examined at a range of magnifications and images captured which were deemed representative of a particular effect. I used the model provided by Pawert *et al.* (1996) to provide a semi-quantitative description of differential effects at the cellular level

C. RESULTS

1. Internal morphology of the larval cranefly

A diagram of the key morphological features of the dissected intestinal tract of the larval cranefly is given in Figure 7.1 and should be consulted in conjunction with the two, whole body, longitudinal sections shown in Plate 7A (i) and (ii). Plate 7A (i) depicts a dorso-ventral section whilst Plate 7A (ii) depicts a lateral section. Features present in these sections include; (a) The buccal cavity with maxillary structures including paired mouth hooks; (b) The bright pink-stained cytoplasm and collagen fibres of the smooth circular muscles associated with the head and buccal area; (c) Fat bodies which stain slightly bluer than the muscle; (d) paired salivary ducts close to the intersection of the eosophagus and foregut; (e) two of the four, blind gastric caeca associated with the posterior end of the foregut, which function to increase the surface are for secretion of enzymes and absorption of fluids (Romoser and Stoffolano 1981); (f) a convoluted midgut which is comparatively long and extends posteriorly; (g) the malpighian tubules which extend from the pylorus at the junction of the midgut and hindgut; (h) the hindgut which has a highly invaginated inner surface and a lattice-like external texture; (i) granulated gut contents present in the hindgut; (j) the thin-walled rectum; and (k) the location of posterior respiratory spiracles.

2. Semi-quantitative analysis of gut ultrastructure

A semi-quantitative analysis of the key ultrastructural differences between control cranefly larvae and those exposed to a range of Cu and Zn spiked soils is given in Table 7.1. Irrespective of the level of exposure to metal spiked soils, tissue fixation was generally poorer in treated larvae. The hindgut in particular was poorly preserved. A lack of cohesion between cellular components in the midgut epithelium was observed for all treated larvae although the extent of the irregularities varied. The cells of the gastric caeca appear fragile [Plate 7B (i)]. Contraction of the basement

membrane from the basal cell membrane occurred throughout the tissues of larvae which had been exposed to the highest Zn metal concentrations [Plate 7B (ii)].

Table 7.1 Tissue-level alterations associated with the internal structures of *Leptotarsus* spp. following heavy metal exposure

Effect	Control	Copper (ppm)			Zinc (ppm)		
		60	120	150	80	120	340
Poor Fixation of foregut	-	(+)	+	+	(+)	+	+
Poor Fixation of hindgut	-	+	++	++	+	++	++
Lack of cohesion of epithelium	-	(+)	++	++	(+)	++	++
Layering of cytoplasm in fat bodies	-	-	++	++	-	++	++
Large and small vacuoles in fat bodies	++	+	-	-	+	-	-
Many small vacuoles in fat body	-	-	+	++	++	++	++
Gut contents present	+	++	++	++	++	++	++

Semi-quantitative results; -, absent; +, occurred occasionally; (+) occurred rarely; ++ occurred regularly

Condensation of the cytoplasm in the fat bodies was common in larvae at higher rates of exposure (120 and 150 ppm Cu, 120 and 340 ppm Zn). The fat bodies in the control state were typically robust and hexagonal in shape with a heterogeneous mix of large and small vacuoles throughout [Plate 7C (i)]. Fat bodies from the larvae exposed to higher Cu and Zn spiked soils appeared to be compressed and layered, with many small, bunched vacuoles throughout the tissue [Plate 7C (ii)].

The presence of gut contents in all sectioned larvae indicated gut clearance was only partial. However, treated larvae had a higher incidence of gut material than did the control larvae. The gut contents appeared as a mixture of crystalline and organic material [Plate 7D].

3. Localization of copper and zinc

The rubeanic acid stain used to localize the presence of copper ions in the digestive tract identified both interstitial granular material in midgut epithelium as well as a component of the gut lumen [Plate 7E (i) and (ii)]. Greenish black staining areas were also located near the basal membrane of the Malpighian tubule [Plate 7E (ii)].

There is some doubt as to whether the dithizone stain was effective in identifying zinc in the larvae. All sections stained weakly with minimal contrast and the consistent effect was an image of the luminal space of the gut [Plate 7F(i) and (ii)]. Differential fixation of the gut is clearly seen between the control state [Plate 7F (i)] and the specimen from the 340 ppm Zn treatment [Plate 7F (ii)]. Darker blue-black material in the gut may be indicative of zinc deposits.

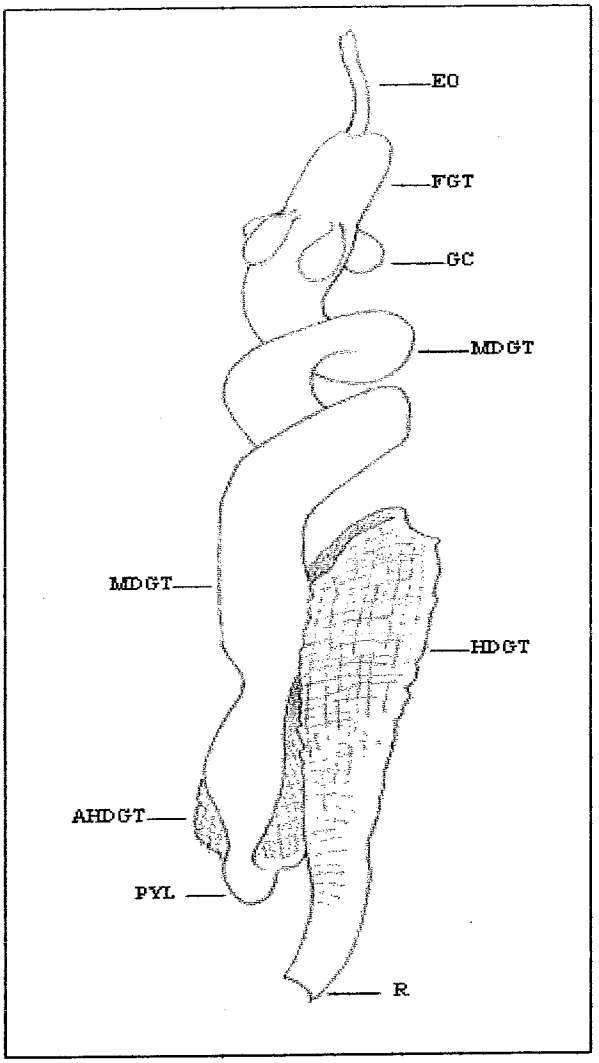

Figure 7.1 Diagram of the cranefly larvae gut. EO = eosophagus; FGT -= foregut; GC = gut caeca; MDGT = midgut; HDGT = hindgut; AHDGT = anterior hindgut; PYL = pylorus; R = rectum.

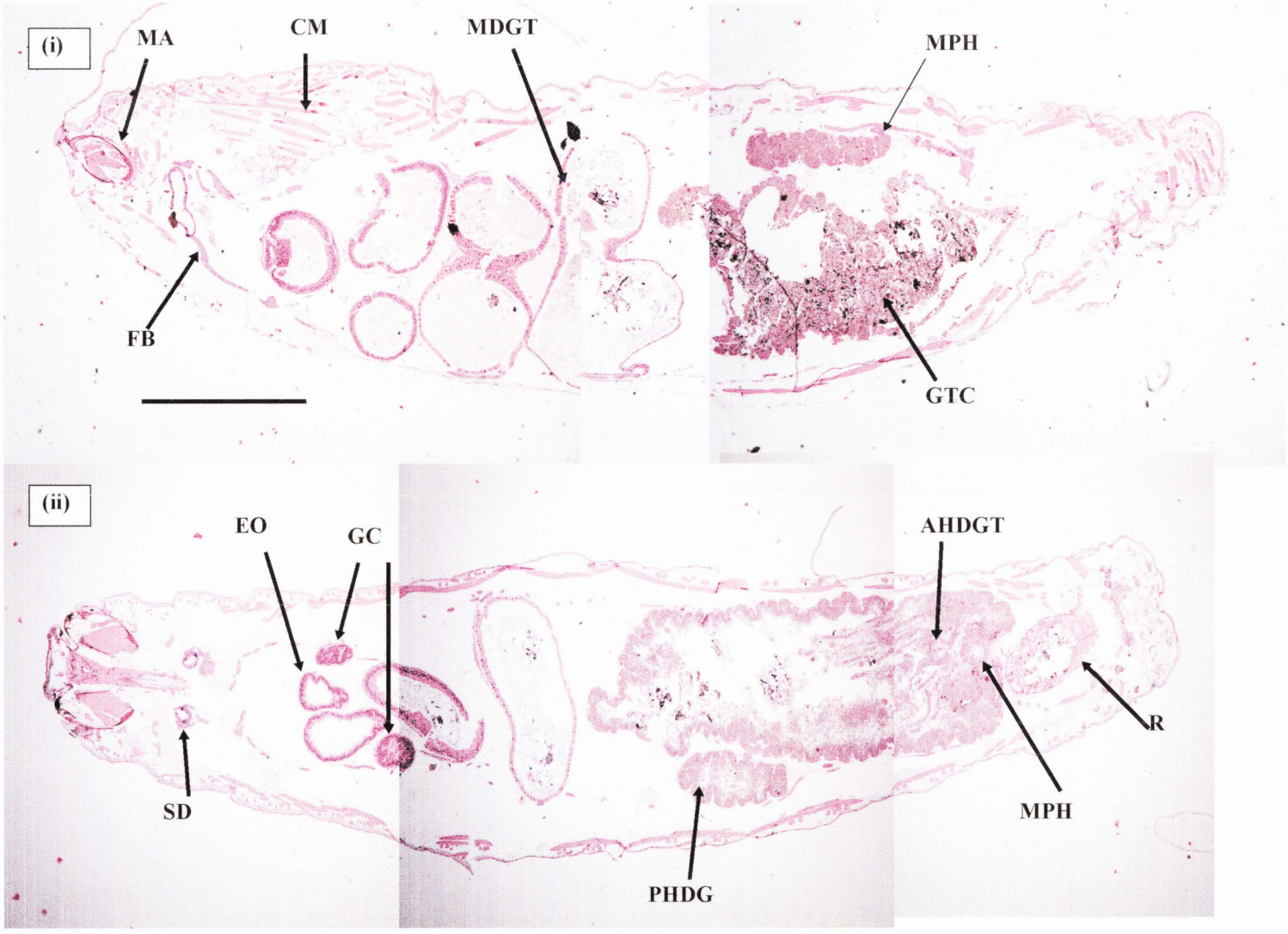

PLATE 7A. Montage of Haematoxylin and Eosin stained light micrographs of longitudinal sections of larval cranefly. Scale bar = 1.72 mm.
(i) Control and; (ii) Specimen exposed to metal salt spiked soil at a concentration of 150 ppm Zn. **MA** maxillary structures and buccal cavity, **CM** circular muscle, **FB** Fat body, **SD** Salivary ducts, **MDGT** Midgut, **GTC** Gut contents, **GC** Gastric caeca, **EO** Eosophagus, **PHDG** Posterior hindgut, **AHDG** Anterior hindgut, **MPH** Malpighian tubule, **R** Rectum.

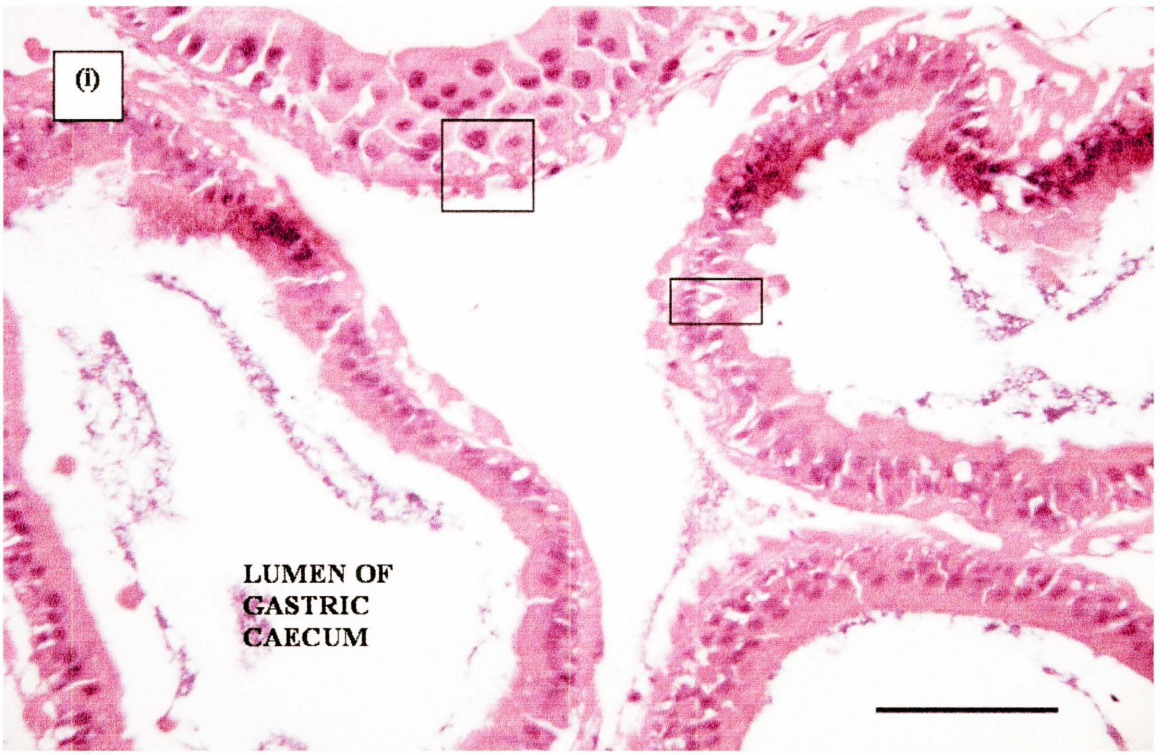

PLATE 7B (i) Haematoxylin and Eosin stained light micrograph of longitudinal section of larval cranefly, midgut and gastric caecum of specimen maintained for 30 days in metal spiked soils at a concentration of 150 ppm Cu. Boxed areas are examples of loss of cohesion. Scale bar = 120µm

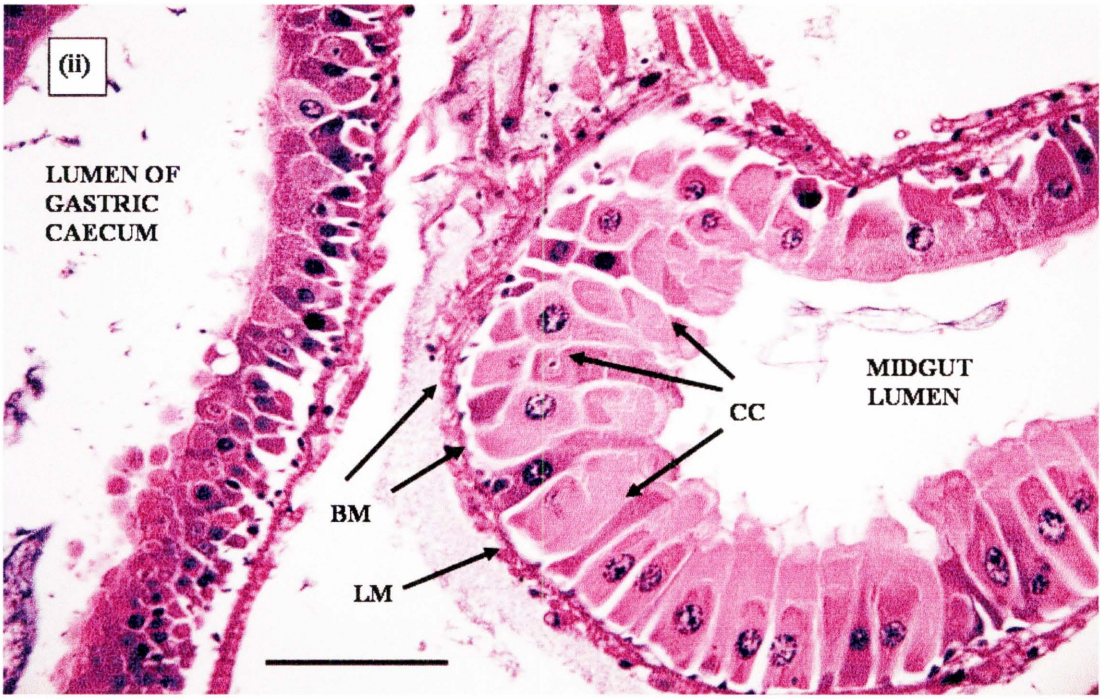

PLATE 7B (ii) Haematoxylin and Eosin stained light micrograph of longitudinal section of larval cranefly; midgut and gastric caecum of specimen maintained for 30 days in metal spiked soils at a concentration of 340 ppm Zn. **LM** Longitudinal muscle, **BM** Basal membrane, **CC** Columnar cells consisting of adjacent lipophilic and cuprophilic cells.

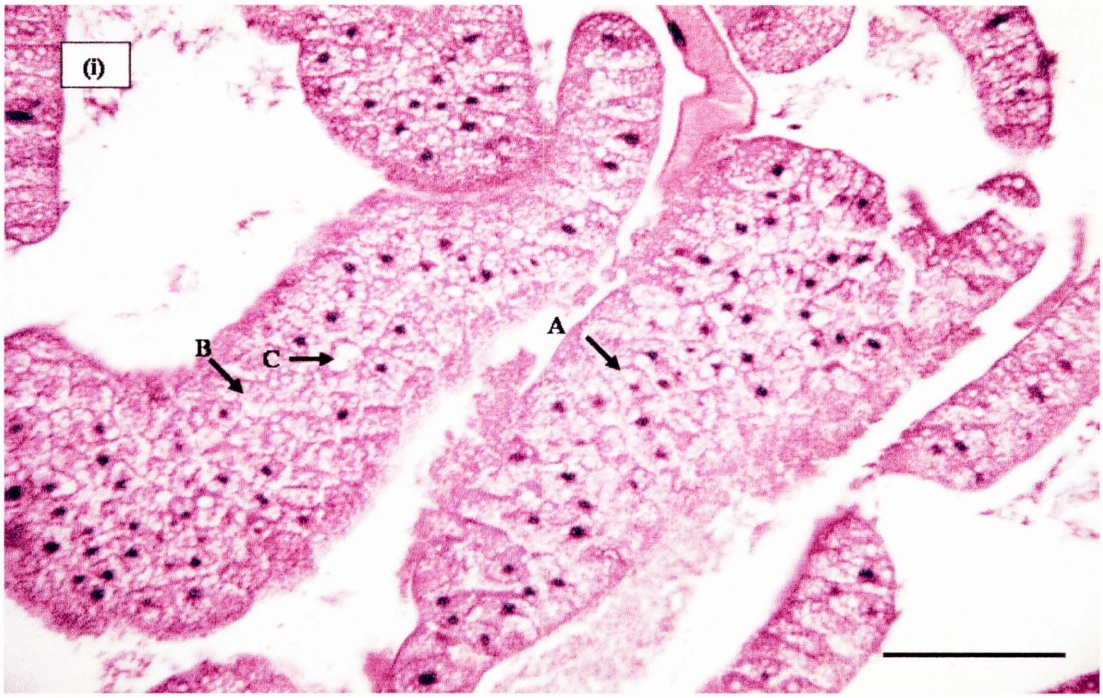

PLATE 7C (i) Haematoxylin and Eosin stained light micrograph of longitudinal section of fat bodies of larval cranefly showing (Control treatment). (A) hexagonal structure of cell walls; (B and C) presence of both small and large vacuoles dispersed irregularly. Scale bar = 120 μm.

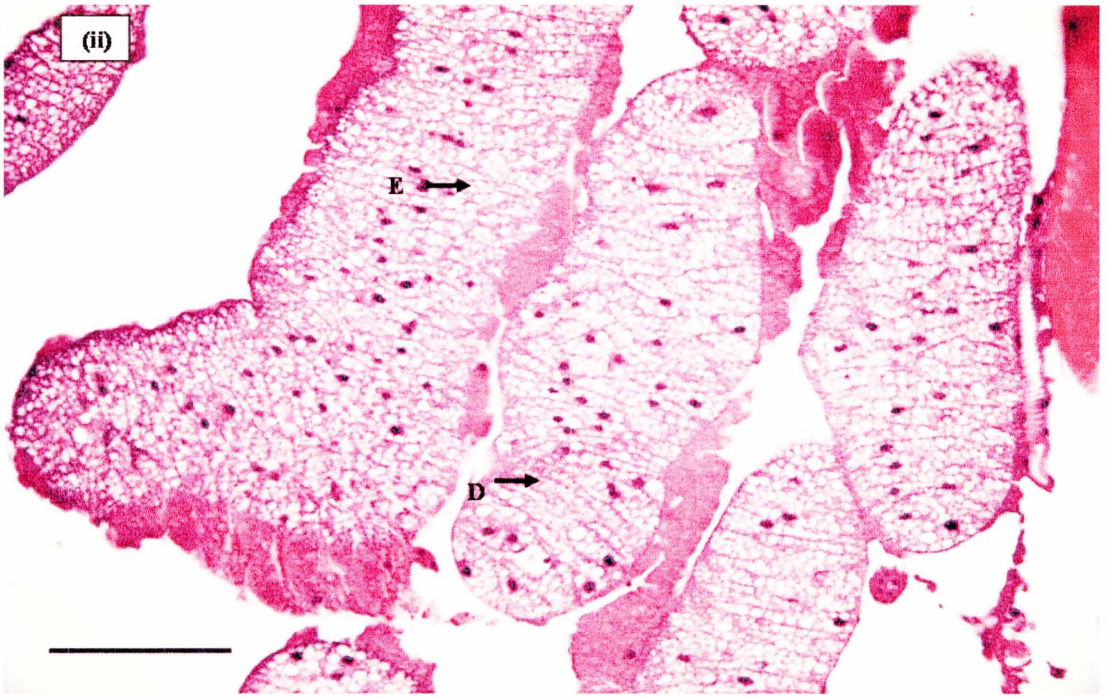

PLATE 7C (ii) Haematoxylin and Eosin stained light micrograph of longitudinal section of fat bodies of larval cranefly; specimen maintained for 30 days in metal-salt spiked soils at a concentration of 340 ppm Zn.
(D) horizontal contraction of cells into "layers" and (E) numerous small vacuoles throughout the fat body.
Scale bar = 120μm.

PLATE 7D. Haematoxylin and Eosin stained light micrograph of longitudinal section of Control larval cranefly foregut showing (A) uncleared gut residue within convoluted foregut; and (B) good cohesion between cellular elements. Scale bar = 120 μm

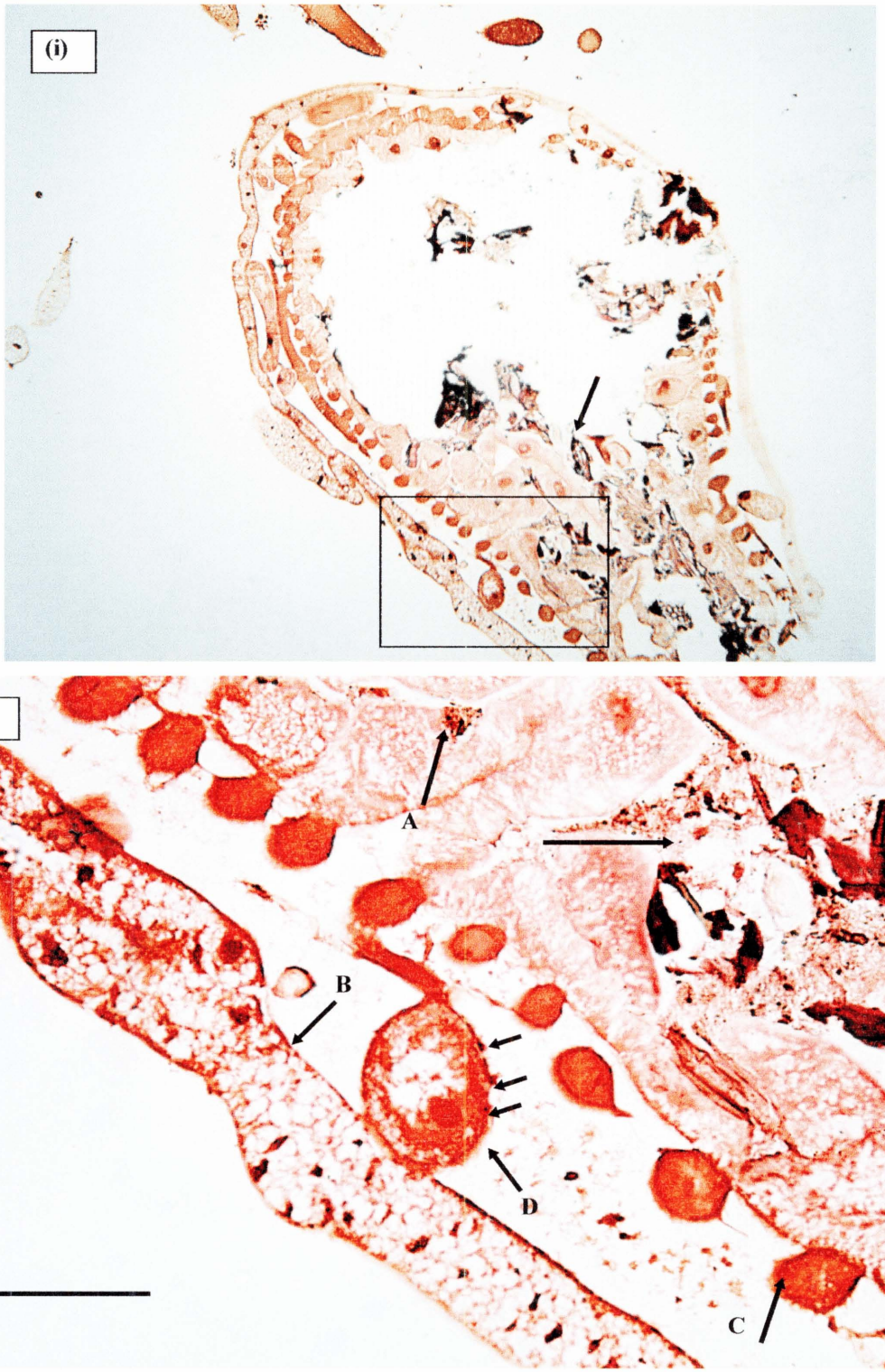

PLATE 7E. Oil immersion light micrograph (rubeanic acid stain) of longitudinal section of larval cranefly posterior midgut. (i) with greenish black copper reactive salts in gut contents (arrowed). Larvae maintained in metal spiked soil at a concentration of 120 ppm Cu for 30 days. (ii) Enlarged boxed section from (i) showing (A) Greenish-black copper salt granules, (B) Fat body, (C) Longitudinal muscle, (D) Malpighian tubule with concretions arrowed.. Scale bar = 80μm

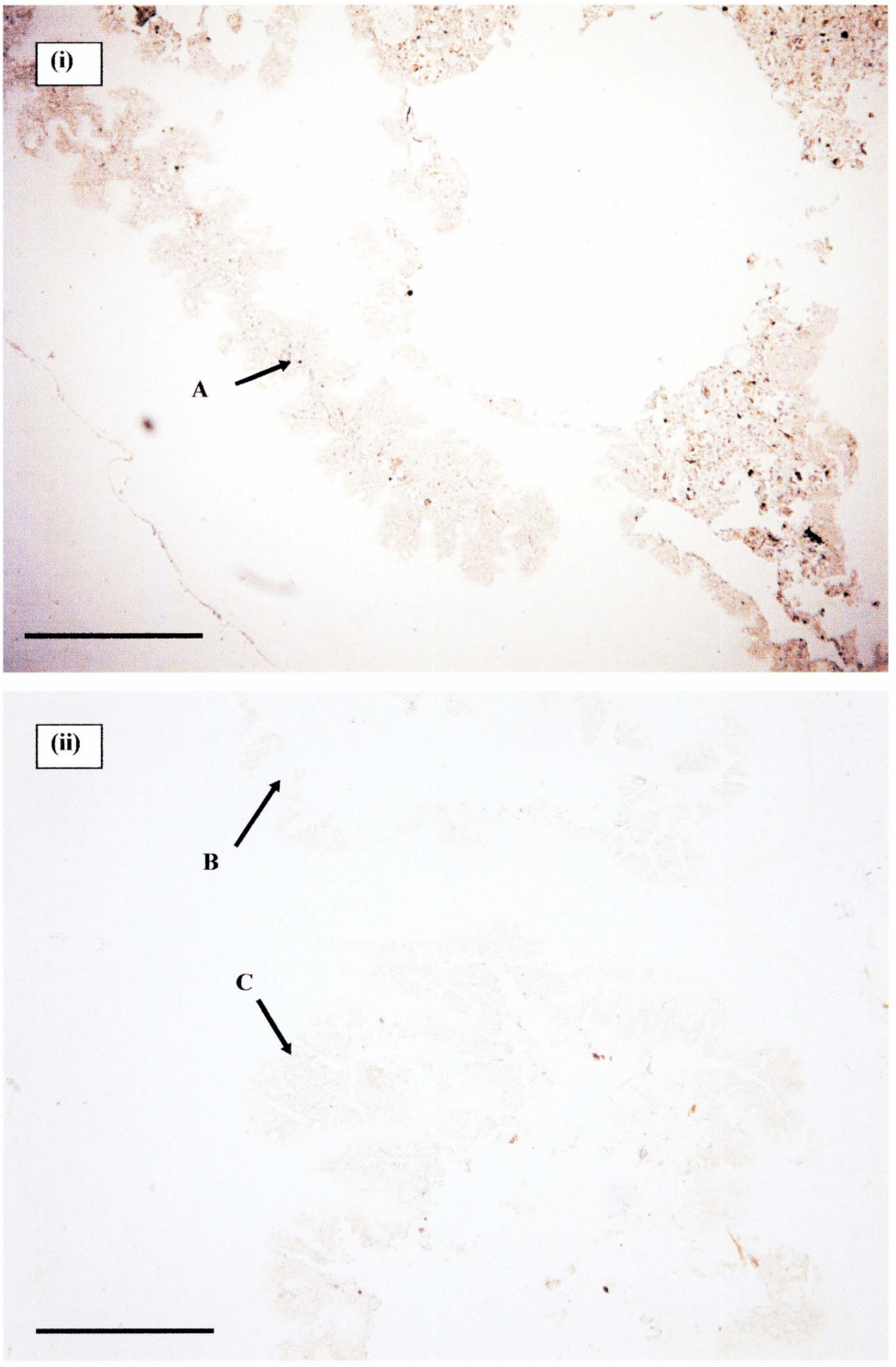

PLATE 7F. Light micrograph of longitudinal sections of larval midgut stained for Zn. (i) Control specimen showing (A) anterior midgut. Scale bar = 100 μm. (ii) Specimen maintained in metals spiked soils at a concentration of 150 ppm Zn showing (B) Midgut and (C) hindgut. Scale bar = 100 μm.

D. DISCUSSION

Tissue fixation

One of the most obvious differences observed between treatment groups was the variability in the effectiveness of the fixative. This was interesting because all control larvae responded better to the fixative than all treated larvae, although the level of fixation in the control larvae was less than optimum. The 8% formaldehyde used as a fixative may have poorly penetrated the orifices and thick outer membranes of the dead larvae. The difficulties posed by the thick outer skin may have been overcome by dissecting out the internal structure.

Better infiltration of the entire bodies may have been achieved by injecting the live larvae with 4% paraformaldehyde fixative behind the head capsule (Brooks *et al.* 1996) which is also likely to have improved the clarity of the photomicrographs for staining by H&E. However, it unlikely to have improved the effectiveness of the metal specific stains, for which alcohol fixation may be preferable to identify, for example, dithizone reactivity to zinc ions (Elmes and Jones 1981) and in some cases, copper ions (Prosi and Dallinger 1988).

Although the outcomes were sufficient to identify a series of ultrastructural differences and evidence of metal accumulation, they are expected to have been improved, had experimentation with both fixatives and stains been carried out, enabling the allocation of larvae to a fixative that maximized the performance of the stains of choice.

Cellular architecture

Substantial differences in tissue and cellular ultrastructure of the midgut were observed between control and treated larvae. Damage to the ultrastructure of the midgut is a more serious situation for Diptera than to the foregut. This is because the foregut intima is impermeable and no substances are absorbed from it into the heamolymph. It is the midgut area where columnar digestive cells, regenerative cells and endocrine cells are present and active in secretion, digestion and absorption.

There was a tendency for dissociation between cellular units, including the contraction of the basement membrane from the basal cell membrane and rifts between columnar cells of the midgut in the treated larvae not observed in the control state. As all larvae were killed and processed simultaneously, it is unlikely that these effects were due to the preparation methodology or are simply histological artifacts. Fractures in sections occur because of chemical hardening during preparation, but this was minimized by using chloroform rather than xylol as a clearing agent.

The observed effects appear to be dose-related, because they occurred less frequently in the larvae treated at either 60 ppm Cu or 80 ppm Zn, than in larvae from higher metal treatment rates. The breakdown in tissue continuity may therefore be a metal-induced effect. However, separation of cause and effect is confounded by the acidity of the test substrate. This is for two reasons. Firstly, the spiked soils were acidified by the metal salts, which may have enhanced the bioavailable fraction of copper or zinc ions for uptake (Speir *et al.* 1999) thereby exaggerating the observed effects. Secondly, ingestion of acidic particulate matter by these geophagic/fungivorous larvae could have altered the pH balance of the gut, upsetting the cellular chemistry and causing degeneration.

The pH of the dipteran midgut tends to be alkaline in the anterior section (pH 7.4-7.6) and becomes highly acidic in the middle (pH 3.3-3.6) and returns to pH 7.6 - 8 prior to the origin of the Malpighian tubules and the start of the hindgut (Waterhouse and Day 1953). Acid gastric juices can alter the species of an ingested metal, thereby rendering that metal more or less toxic (Donaldson and Barreras 1966, Doughtie and Ranga Rao 1984). Changes to the pH of the gut are expected to alter cellular functioning which in turn affects an organism's ionic and osmotic regulatory mechanisms.

Transmission electron microscopy has previously identified altered cellular architecture in midgut epithelial cells following exposure to zinc, in the collembolan *Tetrodontophora bielanensis.* Fenestration of the endoplasmic reticulum (ER) and the presence of ER-derived vacuoles throughout cells occurred in conjunction with condensation of the cytoplasm in specimens exposed to high concentrations; portions of the microvillus border also appeared destroyed (Pawert *et al.* 1996).

Evidence for growth limitation

With the exception of the 340 ppm Zn treatment group, all larvae in the experimental groups were shown to gain biomass during the exposure period. However the significant differences in biomass attributed to treatment effects indicate growth was limited. There are a number of physiological explanations for the observed growth limitation.

There is convincing evidence that treated larvae had utilized stored energy reserves during the exposure period. The fat bodies, which are dynamic structures involved in homeostasis, were observed to differ substantially in appearance to the control state. Condensation (the conspicuous layering of the cytoplasm) and altered vacuole arrangement, suggested the treated larvae may

have metabolized stored glycogen and lipids, which constitute more than half of the fat body contents.

Regression of the fat bodies is common in insects if resources are insufficient (Chapman 1969). For example, under conditions of food deprivation, the hepatopancreas of the isopod *Armadillium vulgare* (viewed by the electron microscope) is dominated by autolysosomes, with lipid inclusions and glycogen either absent or present only in very small amounts (Storch 1984).

Because of the acidity of the spiked soils, localized digestive processes, which are pH dependent, may have been impaired by the ingested acidic substrates. Ingested food materials may have therefore been available for cellular growth but unable to be effectively metabolized because of gut chemistry. The observed structural damage to the midgut epithelium could also reduce the relative effectiveness of extracellular digestion and the subsequent absorption of nutrients. A lack of cohesion between cell types could impair the efficient transport of metabolized products.

Growth limitations may have occurred through the antagonistic effects on cellular metabolism. An example is the binding of ingested bioavailable metals to other elements in cells, which was demonstrated for the gastropod *Arion ater.* The copper that accumulated in calcium cells was excreted from the digestive epithelium by releasing the calcium cells or portions thereof, thus reducing the amount of calcium available for cellular processes (Marigomez *et al.* 1998).

Localization of copper and zinc

Localization of zinc in larval tissues was poor. Surplus dietary zinc was expected to cross the epithelial brush border membranes and be stored within cells in the midgut (Ahearn *et al.* 2001). The photomicrographs only provided images of the luminal surface of the mid and hindgut with poor contrast, thereby limiting their interpretation. It is possible that the process of staining sectioned tissues to identify sequestration sites was confounded by the loss of metal characters due to baseline histological procedures (Grelle and Descamps 2000).

Factors contributing to the inconclusive results for zinc could have included (i) the use of a sub-optimal fixative, (ii) an inadequate method of detection and viewing zinc, (iii) the possibility that the zinc present was tightly bound and unavailable for combination with dithizone, (iv) the loss of available zinc ions during processing if they were lightly bound and water soluble (Elmes and Jones 1981). The sensitivity of different invertebrate tissues to dithizone staining has been noted (Soto *et al.* 1996).

Whilst more sophisticated methods, such as autometallography, can effectively pinpoint and quantify metal accumulations, staining remains an economic and generally reliable tool. For these reasons, rubeanic acid and dithizone were expected to sufficiently identify tissue-level localization of copper and zinc. However, future investigations to localize zinc in metal stressed dipteran larvae may benefit from trialing and perfecting the staining methodology.

The rubeanic acid stain used to demonstrate copper was effective. The pockets of greenish-black material predicted to be present (Pearse 1972) were observed, localized in the interstitial space of the midgut and within the gut lumen. This suggests that some copper had been sequestered, whilst other reactive ions may have been linked to the uncleared gut contents or alternatively, released as part of the detoxification pathway.

Although histochemical techniques offer a relatively inexpensive means of detecting metal compounds in cellular material (Borovansky 1997), more sophisticated technologies enable the generation of quantitative information through, for example X-ray microanalytical mapping (Hopkin 1989). Ultrastructural changes can also be quantified by, for example, digital analysis of differential thicknesses of epithelial tissue (Marigomez *et al.* 1998). A subsequent study utilizing craneflies would benefit from using more sophisticated techniques in conjunction with more rigorous control of potentially confounding factors.

Gut clearance

The histology indicated that gut clearance was partial in all larvae and substantially reduced in treated larvae. There were a number of possible explanations for this outcome. Firstly, the protocol of 48 hours clearance with repeated changes of filter paper, until it was no longer soiled, may have been an insufficient period. However, as there was a treatment differential, it is unlikely that the protocol was completely culpable.

An alternative or supplementary explanation may be a physiological response to food deprivation. However, it is unknown which processes function in these dipteran larvae to activate peristalisis. The larvae may rely on the osmotic pressure of the food or the haemolymph for efficient throughput. This has been demonstrated in the cockroach *Periplaneta americana* (Blattodea) (Davey and Treherne 1963a, 1963b) and studied in the adult blowfly *Phormia regina* (Diptera) (Gelperin 1966). Alternatively, the passage of food may be either myogenic or hormonally influenced (Romoser and Stoffolano 1981). A clearer understanding of the processes mediating

peristalisis in these larvae would be valuable if the family is explored further in laboratory manipulations.

Incomplete gut clearance could point to physical malfunction of the midgut because of ultrastructural damage. This is unlikely to be a complete explanation, because of the observed differential between control and treated larvae. However, it is intuitively appealing for the treated specimens, because a recent autometallography study using the earthworm *Eisenia fetida* showed soil was retained in the digestive tract of gut-cleared, cadmium-treated specimens, but not in the gut-cleared, control specimens (Grelle and Descamps 2000).

It therefore remains unclear as to what caused the retention of gut material, especially in the hindgut of treated specimens. It is likely that a combination of effects compromised normal gut activity in all specimens. Treated larvae, however, may have had additional structural and chemical difficulties which acted as a multiplier.

Gut content evacuation is a recommended process where organisms are to be used in the assessment of environmental contamination (Cain *et al.* 1995). However, in laboratory manipulations where WBMC is used as a metric of effect, the digestive physiology of specimens needs to be considered so that results are not confounded. Furthermore, correlation between the WBMC of wild and laboratory-reared populations should be mindful of possible discrepancies due to variable gut contents, gut clearance and materials retention (Brieger *et al.* 1992, Wilczek and Migula 1996, Devkota and Schmidt 2000).

The whole body metal concentrations (WBMC) reported in the previous chapter therefore need to be viewed with caution. If gut contents were only partially evacuated during the clearing process, the WBMC quantified by atomic absorption spectrophotometry would also have included a fraction derived from undigested material. In future studies using cranefly larvae, it may be useful to use a growing medium, such as nutrient-supplemented agar, as a clearing agent. Metal residues in this medium would need to be quantified. In order to establish how well the gut is cleared in a specified time frame, it may be useful to digest the exuded material, analyse by AAS and factor this value into the WBMC for the different levels of exposure tested.

Conclusions

The exposure of cranefly larvae to metal spiked soils can be linked to growth limitation. The most convincing support was found in the comparison between the fat bodies of treated and control

larvae. Exposure to 120 ppm Cu and 120 ppm Zn appeared to be the minimum concentration at which layering and condensation of the cytoplasm occurred. Ultrastructural differences in the midgut of all treated larvae also point to physical damage to the gut tissues. However, it is not possible to directly attribute cause and effect to the bioavailable metal, as the extremely acidic test conditions may have been influential in damaging the epithelium and basal membranes, thereby impairing digestive capacity. These outcomes demonstrate effectively the impact of a highly acid environment on metal bioavailability.

CHAPTER EIGHT

SUMMARY AND GENERAL DISCUSSION

A. INTRODUCTION

The three themes underlying the research reported in this thesis were biodiversity, biosolids and bioindicators. The main intent of this thesis was to quantify the relationship between arthropod biodiversity and representative *Pinus radiata* planted forests in mid Canterbury, New Zealand. The impacts of a biosolids application programme on biodiversity in these forests was tsubsequently examined. Selected heavy metals present in biosolids were then examined in a toxicology test using a novel and ecologically relevant bioindicator organism. The rationale behind the study was to provide forest managers with tangible reasons for the inclusion of invertebrate biodiversity as a factor in future site management decisions. The thesis reports from both applied and theoretical perspectives at sequential scales of effect, from community-level to species-level and ultimately tissue-level.

The main hypothesis for the thesis was that biosolids applications represent a novel disturbance event in the forest, with the capacity to alter local invertebrate biodiversity. This is because the physical structure and chemical components of the biosolids have the potential to mediate abiotic changes within the habitat which are significant for some invertebrate species. Biodiversity is a holistic concept, encompassing all biota, both introduced and indigenous. It was contended that if biodiversity is altered to a point at which the resistance and resilience of the biotic components are compromised, both soil health and forest productivity could be placed at risk.

The entomology of the soil and litter species under *P. radiata* has traditionally been ignored for its contribution to local biodiversity and ecosystem processes. This point was highlighted in the 1996 report prepared for the Christchurch City Council, which was integral to the granting of a resource consent to apply biosolids to selected planted forests in mid Canterbury. The ecological effects assessment plan clearly addressed the pertinent *abiotic* parameters of concern, but summarily dismissed *biotic* aspects, because "...ecosystems within pine plantations are lacking in diversity (and) at the proposed application rates, livestock, plants or wildlife are unlikely to be adversely affected..." (Anon. 1996).

It was this narrow perception of biodiversity which prompted the research presented in this thesis. It is contended that the 1996 management plan failed to address a key tenet of biodiversity, which is to preserve the ecological integrity of systems through the protection of the fauna contributing to ecosystem function. The forestry industry is reliant on these functions (eg. decomposition and nutrient cycling) for yield stability and thus the conservation of the soil and litter fauna may have important economic repercussions. In order for managed ecosystems to be sustainable, ecological considerations must rank along with social and economic concerns. This is the "triple bottom line" reporting methodology adopted by leading resource management agencies.

In this thesis, the arthropod assemblage associated with the *P. radiata* planted forest provided a tool, with which both applied functions and theoretical concepts were investigated. These approaches are complimentary and were used to examine biodiversity and organizational complexity at a range of scales and varying grain. Each of the research objectives set for this thesis were met by either field surveying, experimental manipulation or visual analysis. However, some of the research questions were answered more satisfactorily than others. Because some research areas remain open-ended, there is variability in the confidence with which conclusions attributing cause and effect can be drawn.

The general discussion presented here is structured around the research questions which form the skeleton of this thesis. Within each of these research areas, the key findings are reiterated and discussed in relation to the extent to which they support or contradict previous studies. Possible reasons for observed differences are offered, as well as the implications of the research outcomes. The limitations of the research are outlined separately and the chapter is concluded with a summary of suggestions for future studies and closed with a final overview statement.

B. REITERATION OF THE MAJOR FINDINGS, IN RELATION TO THE RESEARCH QUESTIONS, REPORTED IN THIS THESIS

1. Invertebrate biodiversity and the P. radiata planted forest

The *P. radiata* planted forests in mid Canterbury offer a habitat and refuge for both indigenous and introduced arthropod species. It is a distinctive and gregarious assemblage, dominated by species sharing a tolerance of human-mediated disturbance. Many of the species present are opportunist colonizers (both indigenous and introduced) with a wide representation across a variety of habitats and geographic locations.

The surveys showed that whilst a core species assemblage was present in these forests, the specific abiotic features characteristic of an age class generated their own unique faunal assemblage in a reasonably predictable manner from the period of establishment through to economic maturity. Abiotic parameters of interest were shown to include microclimate and inferred from tree architecture and forest floor structure. Age-related management, such as pruning or waste thinning were predicted to influence the composition of the invertebrate community because these activities modify microclimate and provide resources for colonizers. Although data loggers may have more precisely monitored stand microclimate, the simple analysis of forest floor temperature effectively identified key site-specific differences.

For example, the most obvious difference between the two stands HR and DB was the insulation afforded the mineral soil by the litter layer accumulated on the DB forest floor. Many of the differences in the seasonal representation of species could be attributed to site microclimate, which supports outcomes reported for both exotic and *Nothofagus* forests by McColl (1997a, 1997b) and is in agreement with the general microclimatic effects and changed conditions noted under *P. radiata* agroforestry regimes demonstrated by Hawke (1994) and shown by Yeates and Hawke (2000).

The description offered for the arthropod assemblage associated with *P. radiata* reiterates that of Johns *et al.* (1980) who proposed the phrase "limited, yet distinctive". Their high country Hanmer-based study noted the presence of many "common" introduced species, but also commented on the infiltration of a number of indigenous species into the old planted forests, aided possibly by the proximity of nearby *Nothofagus* forest. It is therefore noteworthy, given the highly modified agricultural landscape which surrounds the mid Canterbury forests and their relative isolation from substantial areas of native forest, that a number of indigenous forest species are a feature of the species assemblage. This interpretation is supported by other New Zealand-based studies, notably Schipper (1996) and Hutcheson and Jones (1999), in which the combined effects of the surrounding landscape and the forestry cycle on invertebrate biodiversity have been shown.

There are repeated examples in the literature which have set to compare the biodiversity of exotic and indigenous forests and the outcomes are unequivocal. Diversity is reduced in the exotic forest. However, this thesis contends that, irrespective of provenance, the basic ecological processes mediated by the invertebrate biota are the same. The notable differences are the level of complexity of the interrelationships between the biotic compartments and the extent to which

specific groups are able to maintain viable populations. Given the expected limited availability of colonizing species, the assemblage in mid Canterbury's forests is all the more remarkable because it demonstrates the aggregation and interaction capacity of a diverse and even disparate community within a relatively short time frame in a biotically simple and highly modified habitat. The interactions between trophic groups was clearly shown to be influenced by an abundance of predatory and phytophagous species. Many of the predatory species, including the adult and juvenile staphylinid beetles, spiders, harvestmen and some myriapods, were ground active and readily trapped in pitfalls. The behaviour of the spiders was quite diverse in relation to their prey capture methodologies, and included species which hunt by sight, sheet web builders and cobweb builders. The phytophagous species had clear associations with understorey vegetation, which is a variable resource dependent on weed control procedures and the forestry cycle itself.

The trophic structure described for the *P. radiata* arthropod assemblage in the study sites reported here differs substantially to that of the malaise-trapped beetles in *P. radiata* stands in Kaingaroa in the North Island of New Zealand described by Hutcheson and Jones (1999). The community-level trophic structure for that beetle assemblage (trapped over four consecutive weeks in mid summer) was dominated by detrivores, reflecting the importance of an abundant debris resource for driving community structure. It is also understandable that the outcomes differ because of trapping methodology and the target invertebrate group.

Furthermore, Allen et al (1995) demonstrated that North Island planted forests often sustain a more complex and diverse under storey than their South Island counterparts. However, irrespective of location, stand dynamics and management practices in combination generate substantial accumulations of carbon on the forest floor over the period of a rotation, providing a resource for detrivorous and fungivorous species. Had the general invertebrate community been sampled in their survey, it is probable that a sizeable and dominant predatory fraction were present to capitalize on an abundance of prey targets.

Hutcheson and Jones (1999) used their detrivorous-dominated beetle assemblage to examine the linkage between biodiversity, invertebrate-mediated nutrient cycling and site productivity. A similar linkage was difficult to extrapolate from the community-level data presented in the mid Canterbury surveys. This is because predatory and phytophagous species are secondary consumers which are too far divorced from the "grass roots processes". However it is not impossible to quantify energy transfer in selected bioindicator species. This has implications for future investigations because the predatory species in particular represent a very examinable

group for which biomass and energy transfer might be quantified. Further studies examining energy flows, rather than the simple abundance of individuals may be rewarding.

The trophic structure described for the planted forests has theoretical relevance and the widely accepted redundancy theory (Lawton and Brown 1994) may well be applied to the invertebrate assemblage. This is because across the sampled age classes, the trophic structure exhibited substantial numerical dominance by predatory species. The loss of one or more predators is not thought likely to impact upon ecological processes (such as prey regulation). This is because other predators will probably be able to maximize their own fitness by utilizing the vacant resource niche. A similar situation is likely for the phytophagous species shown to occupy a secondary position in the trophic structure. However, at the lowest tier, the amount of functional replication declines to the point where only a few species appear to be represented.

For example, it was shown that the myceto/geophagic tipulid larvae, represented by four indigenous species, are abundant in stands supporting accumulated litter and also the sole representatives of their functional group. Tipulid larvae are successfully exploiting the planted forest habitat and probably contributing to fungal regulation, the inoculation of fungal material through the soil space and because they burrow, soil aeration. The historical loss of soil invertebrate species diversity across much of New Zealand's arable landscape is well-recognized. Both Yeates (1991) and Lee (1961, 1985) have provided a review of these losses. Whilst it is unknown to what extent tipulids populations were present in the past in the study site locality, they may (at least at the local microsite scale) currently represent a compensatory mechanism providing ecological services more traditionally associated with the absentee earthworm fauna.

Perhaps one of the most distinctive differences noted for the mid Canterbury forests was the absence of earthworms irrespective of stand age. In a North Island agroforestry trial at Tikitere, Yeates (1988) gave estimates of average earthworm populations of 283 per m^2 in plots containing 50 *P. radiata* 13 years after planting. However, it was noted that earthworm abundance was substantially reduced compared with estimates made approximately 10 years previously. That paper also drew attention to the work of both McColl (1974b) and Styles (1967) who did not document earthworms in their surveys. Whether the lumbricids were either ignored or not present at their sites remains unknown. Interestingly, unspecified dipteran larvae were included in their surveys, so it is possible that this group represents an ecologically important fraction of the litter biomass within forestry systems. The lack of family level designation for these dipterans limits further speculation.

It has become something of a cultural habit to perceive indigenous fauna as delicate and vulnerable to disturbance. However, given the indigenous representation within the *P. radiata* habitats surveyed in this thesis, it is clear that a fraction of the biota is very tolerant of habitat disturbance and modification. This resilient fraction makes an important contribution tolocal biodiversity. Prominent examples include the two Canterbury endemics, *Hemiandrus* sp. and *Taieria erebus,* which had not previously been recorded from this locality but are regularly found in association with the farmland and urbanized areas of nearby Christchurch and Banks Peninsula. It is useful to compare their occurrence with an even more resilient and widely distributed indigenous spider, *Lycosa hilaris,* which actively capitalizes on modified habitats.

The surveys also identified a handful of indigenous spider species represented by a very few individuals which are maintaining marginal populations in the planted forests. Examples include *Lycosa* n.sp. and *Oxyopes gracilipes*. Similarly, the occasional presence of poorly known coleopterans, such as the weevils Curculionidae RTU1 and RTU2, and the fungus beetles Corticaridae RTU and RTU2, indicate resources in the planted forest are sufficient to support limited populations. However, it is unknown whether these populations are restricted because of habitat and resource suitability, competition, the shortage of nearby founder populations, or poor mobility. Even less well understood are the biologies, life histories and identities of these small and cryptic species. This situation reiterates the on-going challenge of taxonomic insufficiency with respect to the New Zealand fauna as noted by Klimaszewski and Watt (1997).

The mid Canterbury surveys showed that introduced species tended to dominate the stands in the early stages of development. As stands progressed towards maturity, indigenous species were found to constitute more than 50% of the ten most abundant species. The proportional representation of indigenous beetles across early and mature age class stands was less than 40%. This is substantially lower than the 61% reported by Harris *et al.* (2000) for malaise-trapped indigenous beetles resident or dispersing through modified pastures in a North Island study. Although this appears a huge differential, it is at least partially explained by the proximity of their sampling site to native vegetation and the fact that their sampling strategy targeted highly mobile species rather than ground active species.

The surveys reported in this thesis have shown that arthropod species diversity in exotic monocultures is unlikely to be limited simply because the habitat is managed and highly modified. It is more likely diversity is limited by their relative isolation and the availability of

founder populations typical of forests. The planted forests represent a series of fragments which are significant physical subsets of a highly modified regional landscape. Modified habitats can and do support a diverse community of species, irrespective of their provenance. It may be timely to move on from the mindset of equating pine plantations with "ecological deserts" as criticized by Allen *et al.* (1995) and examine the context in which the existing biotic structure effectively provides ecological services beneficial for ecosystem health, site productivity and local biodiversity.

2. A model of invertebrate diversity in the P. radiata habitat

The spatial heterogeneity theory predicts a positive correlation between species diversity (H') and the complexity of a habitat. The simple predictive model developed for the soil and litter fauna associated with mid Canterbury's *P. radiata* forests suggests invertebrate species diversity (H') increases with the age class of a stand. This increase was best described by a logarithmic trend line ($r^2 = 0.323$), rather than the linear model predicted from the spatial heterogeneity theory.

The pattern of rapid increase in species diversity (H') in the initial 10 years of stand development mirrors that found for plant species diversity (H') in North Island planted forests by Ogden *et al.* (1997) and Allen *et al.* (1995). It also reflects the patterns observed for arthropod species in Texas pine plantations by Bird *et al.* (2000). It is likely to reflect the simple process of rapid colonization and utilization of a vacant niche by adaptable and behaviourally plastic generalists. The logarithmic model suggested that the rate of species accumulations tended to slow as a stand progresses towards economic maturity. It remains unclear whether the observed effect is due to species saturation of the habitat or constraints attributable to microclimate, nutrient bottlenecks or lack of founder populations. This is a rich area for further investigation.

The proposed model is helpful because it can be exploited to manipulate directional shifts in diversity through specific management options. For example, populations of fungivorous beetles associated with wood decomposition may be retained throughout a site after harvest by leaving intact the forest floor and harvest by-products, rather than windrowing into heaps which may be left to rot or subsequently burnt. It is possible that one of the most poorly recognized sources of invertebrate diversity within a stand is provided by the windrow habitat.

The proposed model does have a limitation. It cannot be said to reflect habitat heterogeneity simply because diversity (H') increased with time. This is because the spatial complexity of the habitat cannot be divorced from either microclimatic effects, the quality, or the quantity of the

resources which constitute the structural architecture. This point echoes the difficulties in interpreting the concept of "heterogeneity" which has been a discussion point for some years. This is because the simple definition "spatial patterns that vary in a systematic way from place to place are called heterogeneous" noted by Ripley (1981) can fracture into complexity as the viewing perspective changes. Thus, the type of spatial pattern observed provides an important reference point (Dutilleul 1993).

The model is in general agreement with the prediction of a positive relationship between habitat complexity and species diversity suggested by Davidowitz and Rosenzweig (1998). The point of contention is the interpretation of the observed response and the subsequent determination of cause and effect. It may be more appropriate to say that the logarithmic shift in diversity (H') with time was a resource-based response. Although other studies have interpreted increases in species diversity as a response to developing spatial complexity (Hanowski *et al.* 1997, Davidowitz and Rosenzweig 1998, Dennis *et al.* 1998), that space needs to supply appropriate resources for colonizing species. Thus, it is the heterogeneity of the resources that increase species diversity, rather than the fact that the habitat is spatially heterogeneous. This interpretation is supported by Marra and Edmond's 1998 study which argued woody debris on the forest floor may contribute to three dimensional spatial complexity, however it is the amount of debris and its proximity that has been shown to promote mite diversity.

Future studies examining the habitat heterogeneity theory in the context of invertebrate diversity in the planted forest should quantify the meaningful structural components of the invertebrate habitat. For this to be effective, an understanding of specific life histories and species biologies is paramount. To minimize the complexity of such an investigation, the indicator species approach using a suite of typical species may prove invaluable.

3. The effect of incremental biosolids applications on the abundance of species in functional groups

No significant alteration attributable to biosolids applications was found in the abundance of species in arbitrary functional groups in the mid Canterbury forests. At biosolids application rates of up to 800 kg N/ha, existing ecosystem processes and local diversity are unlikely to be affected, at least in the short-term. Although there were indications from the data that biosolids treatments at the 400 kg N/ha rate had a positive effect on arthropod abundance, it was shown that this increase was due mostly to the presence of collembolans. Further analysis showed that collembolan abundance was highly variable within replicates and treatments.

The outcomes do not, however, discount the possibility of long-terms effects from repeated applications, as evidenced from overseas research (Krogh and Pedersen 1997, Andres 1999, Bruce *et al.* 1999). Neither does it discount the possibility that altered community structure could be positive in terms of enhancing diversity and ecosystem processes. The important point raised by this research is that in order to satisfy the issue of sustainability it is necessary to adopt the precautionary principle and maintain effective monitoring programmes. The thesis was valuable to that end because it provided a suite of indicators suitable for future assessments of ecological effects.

The lack of a significant effect of biosolids applications on species abundance in functional groups was unexpected, because biosolids represent a high input nutrient pulse with the capacity to change both the energy budget and the physical habitat. It is well known that nutrient fluxes are key mechanisms driving community change. A variety of factors could have obscured evidence for a significant effect. Biosolids are slow release fertilizers and any observable changes may be slow to diffuse through the system (Bourke *et al.* 1997). Therefore, the 6-month time frame may have been too short, given that other biosolid-oriented studies registering positive community-level responses have been based on several years of accumulated data (Prescott *et al.* 1993, Larsen *et al.* 1996).

Nutrients may also have been immobilized by fungal and microbial populations, as has been shown by Treseder and Allen (2000). Other possible explanations for no observed effect at the community scale include the relative insensitivity of the community to enrichment and disturbance and the buffering capacity of the soil environment (Setälä 2002). One intuitively appealing reason is that the invertebrate assemblage is largely unperturbed, tolerant and insensitive to disturbance. This is appealing because there is ample evidence that a substantial proportion of the arthropod assemblage surveyed enjoy a widespread distribution in a range of modified habitats.

A lack of effect may have been due to the level of analysis employed. Changes in the abundance or biomass of individuals within species may have been a more sensitive parameter and could be recommended for future studies. An abundance/biomass approach was used to good effect in assessing the responses of ground-dwelling arthropods under different forest management practices in North America (Greenberg and Thomas 1995).

Finally, any community-level analysis is likely to be confounded by ecological "noise", which is a characteristic of community studies in which a few species are represented by a many individuals and many species are represented by a few individuals (Giller 1984). One example of noise in the initial characterization survey was the presence of several dipteran families represented by a number of species and a few individuals, most of which had no known dependent biological relationship with the forest habitat. This was based on the absence of respective larval stages from the litter and soil. In situations like this it maybe logical to exclude them from the analysis. However, unless it can be demonstrated that they have no interaction with other biota in the planted forest ecosystem, for example, as prey species for some of the web-building spiders, it is not acceptable to exclude them.

"Noise" is effectively eliminated by reducing the target group of species under examination. A number of other studies have successfully identified changes in the structure of communities in response to biosolid applications by concentrating on specific groups or orders, such as eudaphic collembolans (Bruce *et al.* 1999, Cole *et al.* 2001) or coleopterans (Larsen *et al.* 1996). A targeted analysis is generally derived from an initial survey to determine ecologically relevant and typical species. One reliable method of statistically determining how representative a taxonomic unit is of a specific site or condition is the ISA methodology of Dufrene and Legendre (1997). This method was used extensively in the research to develop a suite of indicator species. It fulfilled the current demand for an economic, rapid and effective assessment methodology suitable for use by non specialists (Oliver and Beattie 1996). Future studies in the mid Canterbury monitoring sites may benefit by using the suggested species developed from the ISA to better elucidate effects of subsequent biosolid applications.

Although a number of possible reasons accounting for the observed lack of effect have been offered, there is of course, no reason to believe that a lack of effect was not a true result. Biosolids applications may not generate community-level effects quantifiable by diversity analysis. This conclusion has theoretical implications for biodiversity assessment, particularly the use of diversity indices. It may be timely to reiterate personal comments by John Hutcheson, who argues that diversity indices fail to advance our understanding of ecosystem processes and reflect the inertia of our thinking systems. For this reason, alternative methods such as visualizing community configurations, affinities and the attributes of component species could be more worthwhile. In view of the DCA ordinations presented, which were remarkable for their lack of site differentiation, it is probable that indeed, biosolid applications did not generate a significant effect on the species assemblage.

4. The effect of incremental biosolids applications on species diversity (H')

Species diversity (H') in the planted forests was not shown to alter significantly in response to biosolid applications at rates of up to 800 kg N/ha. This research question remains very open-ended and it is difficult to draw conclusions from it. Despite the very distinctive differences in stand architecture and forest floor microclimate at the two sites, Sorenson's Index (Sim.) suggested a low turnover in the species assemblage between plots at both sites, indicating reasonable agreement in the similarity of the arthropod assemblage, irrespective of treatment rate or site. The sampling strategies employed highlighted the variability in catch composition within plots, which is likely to have influenced the statistical outcomes.

Further confounding the issue were the very wet weather conditions during and immediately following the applications. As a best guess, site-specific conditions could have altered the physical structure of the forest floor surface making it more or less suitable for different species.

Although other studies have shown species diversity of selected groups to increase in sewage sludge fertilized plots, there is no evidence in the literature of species diversity differentials according to the stage of development of a forest stand receiving biosolids. This area represents a poorly understood and under-researched area worthy of further investigation. Because the differential response could be indicative of an ecological difference which is site-specific, it may be more clearly defined by selecting relevant indicator species from the ISA for re-sampling of the sites. Factors worthy of consideration in such a study could include both physical and chemical effects which are either directly or indirectly mediated by the biosolids.

5. The effect of incremental biosolids applications on the abundance of cranefly larvae

Physical disturbance to the habitat potentially introduces adverse conditions which limit individual success, place species at risk and threaten local diversity. Negative effects at the species-level may go unnoticed because they are obscured by the community-level scale of analysis. A case in point are the indigenous cranefly larvae present in the mid Canterbury planted forests.

Biosolids applications present a physical risk to at least four species of myceto/geophagous larvae In the field experiment presented in Chapter Five, biosolids applied at the lowest rate (400 kg N/ha) resulted in a significant reduction in the abundance of endemic larval craneflies (Diptera:

Tipulidae). This was attributed to the change in the physical interface between the litter and the surface which limits the passive filtration of aerially-dispersed eggs into the needle litter.

Of the three indigenous cranefly species present in forests in this locality, *Leptotarsus zeylandiae* is likely to be the most at risk species because of the physical effects of biosolids. This is because the female is brachypterous and disperses eggs within the immediate vicinity of her emergence from the soil, unless transported off to other locations by the strong-flying male. If passive filtration of eggs is limited by the percentage cover of the biosolids, then it is likely this species will be limited and local biodiversity will be reduced.

Apart from the concerns for local biodiversity, this outcome has relevance to the rate and method by which the biosolids are applied and how a site is subsequently managed. If biosolids are physically incorporated into the soil substrate using deep-row application, the crusting effect is virtually eliminated. Deep-row application is particularly beneficial for tree growth because it can be used to place the nutrient source in the specific root zone areas prior to planting (Sikora *et al.* 1980) and avoids surface crusting which is likely to have microsite effects on the exchange of soil gases and soil chemistry. However, deep row application is only feasible in the post-harvest-period prior to planting or when the trees are very young. It probably has more applicability in the nursery bed situation. Clearly, there are substantial gaps in our understanding of the physical effects of biosolids applied as a layer, on soil processes, the soil community and biodiversity as a whole.

Biosolids also mediate a chemical effect on the soil substrate. This was effectively demonstrated by the significant interaction between treated and untreated pairs of secondary plots in the caged experiments in the field. However, it is difficult to attribute cause and effect because necessary parameters, such as the lateral movement of nutrient-rich leachates which may have affected microbial activity and fungal growth were not measured. This finding remains unresolved. At a best guess, it may be related to the lateral permeation of fungal hyphae through the soil and litter, such that conditions in one microsite may have an effect at another location because the material has been transferred through the hyphal network. A simile which may effectively describe this idea is the effect of systemic herbicides on ground spreading and creeping weed species. There is substantial scope for further experimentation on fungal growth in response to biosolids in the field.

One of the important implications of this experiment was the identification of the level of approach necessary to generate a significant effect on an ecologically relevant organism. Previous analyses had failed to identify a community-level effect of incremental biosolids. The species-level approach utilized known biology and behaviour in a controlled field trial, which effectively minimized the doubt that occurs where laboratory-based outcomes are extrapolated to field conditions. For this reason, confidence in the outcome is very high.

The results support those of Hoevermeyer (1999), who examined the emergence abundances of Diptera from sewage sludge treated fields. He maintained that the use of total Diptera an/or dipteran suborders were insufficient descriptors of the soil dwelling dipteran communities, and advocated the use of larval abundance and biomass data.

Biosolids applications at rates exceeding 400 kg N/ha are expected to reduce larval cranefly abundance and pose a threat to local indigenous dipteran diversity where those species are normally abundant. However, given that at least four species co-exist in the soil and litter habitat and belong to the same functional group, it is likely that functional redundancy will compensate for the loss or decline of some of these species.

6. Cranefly larvae and their contribution to soil physical processes

The laboratory-based experiment designed to determine whether there was a proposed linkage between tipulid larval abundance and their capacity to contribute to soil aeration was convincing in its lack of effect. This experiment was conducted because cranefly larvae had been shown previously to be an "at-risk" group in forest habitats receiving biosolids, but it was unknown as to whether the ecological value of these larvae could be quantified. If shown to influence the physical character of the soil at the microsite scale, which is of particular importance for root penetration and soil gaseous exchange (Sands and Bowen 1978, Ruark *et al.* 1982, Glinski and Lipiec 1990) a case justifying their inclusion as significant elements of site biodiversity could be argued.

The laboratory conditions provided a reasonably realistic physical habitat for the larvae. The larvae were observed to exhibit a very rapid negative phototaxic response when placed on the surface of the experimental soils. The experiment was set to operate at soil matric potentials that are close to field capacity and therefore moisture was readily available. The volumetric water content of the soil (44% at 0.9 g cm^{-3} ρ_b and 64% at 1.1 g cm^{-3} ρ_b) corresponded to porosity values of 55% and 4 % respectively. These values compare favourably with typical New Zealand

soils, where 53-57% is standard for a sandy loam/silt loam and 37-47% is standard for a silt loam/silt clay loam (McLaren and Cameron 1996). It was, however, possible that the 30-day experimental period was too short in relation to the actual time spent by the larvae under natural field conditions (11 months) in the soils. The observed lack of effect may have been due to an insufficient time to generate an effect. Alternatively, larvae may lack the capacity to generate an effect.

Perhaps one of the most important outcomes of this research question is theoretical. This is because of the potential linkage with the redundancy hypothesis and historical aspects of the invertebrate assemblage. Prior to human-mediated habitat modification, the xeromorphic yellow-grey earths of the Canterbury Plains are believed to have harboured indigenous, surface feeding earthworms (Megascolecidae) (Lee 1959) and may have also supported dipteran larvae, especially where forest provided shade and an organic litter layer.

Both of these shallow feeding, lateral tunneling species are typical of fauna which have evolved in a forest ecosystem, where a shallow litter overlies mineral soil. Human-mediated effects have altered both the vegetation and faunal assemblage, reducing the competitiveness of some species, such as the indigenous earthworms and rendering sites unsuitable for related introduced species. Some tolerant invertebrate species, such as the craneflies, may now be the sole representatives of this functional group. These species may well constitute a redundant element. In the absence of an earthworm fauna under *P. radiata,* are the ecological services that may have been provided by both earthworms and cranefly larvae, sufficiently compensated for now by cranefly larvae alone?

The experimental evidence presented in Chapter Five failed to show a significant contribution by the larvae to air filled porosity. However, this expectation may have been unrealistic for the shallow feeding larvae. This is for two reasons, both of which are related to soil bulk density. Firstly, the Lismore silt loam soil used in the experiment is typical of the mid Canterbury locality, being lightly textured (> 75% silt, < 50% sand, < 25% clay) with a naturally low bulk density (D.S.I.R. 1968). Invertebrate movement in these light soils soil may create porous space, but the displacement of particles brought about by horizontal or vertical movement may only be temporary. As an animal shifts the voids may be readily filled and even compacted. This effect is supported by van Rhee (1969) who maintained that under some conditions, invertebrate activity may only be responsible for the redistribution of pore space, rather than an increase in the total soil pores. Secondly, it is unlikely that the historic biotic assemblage encountered soil compaction of a magnitude common today in the heavily mechanized land use activities typical of this region.

For this reason there is no reason to think that the historic faunal assemblage contained species which were active soil aerators or vegetation dependent on the ecological services of such organisms.

In view of the experimental results, it may be concluded that the ecological value of cranefly larvae in the planted forest habitat is directly associated with the regulation of organic and fungal biomass at the interface of the mineral soil and the forest litter and indirectly associated with nutrient recycling and the physical properties of the soil. Any physical alteration of soil structure is limited to a redistribution of soil pore space, which can have important consequences for biodiversity in the litter habitat. This is because the pore sizes within litter increases the structural diversity of the litter layer and influences the vertical distribution of the mesofauna, particularly mites and collembola (Teuben and Smidt 1992). They are also likely to be active in translocation of microbial material from the litter to the mineral soil via frass, which is further utilized as a food resource by detrivorous microarthropods.

The implications of these conclusions are of both theoretical and entomological interest. The historic reduction in edaphic species with a geophagic functional association in the planted forest habitat in this locality, has left the cranefly larvae as remnant representatives. It is unknown whether compensatory mechanisms, such as an increase in cranefly species biomass and abundance have effectively made up for the earthworms losses, which would support the redundancy hypothesis. What is certain is that further losses of some cranefly species and a decline in the abundance of others will be incurred if biosolids applications are applied to stands in which litter has accumulated. These losses can be confidently predicted because the biology and behaviour of the at risk species is known.

7. Bioindicators: The response of species to a contaminated habitat and support for the "scope for growth" hypothesis

The larval cranefly *Leptotarsus* spp. was shown to be an effective and sensitive indicator of chronic metal contamination. Larval growth after 30 days was significantly lower for both 60 ppm Cu and 80 ppm Zn treatments than for the control group. It may be argued that this response is too sensitive, given that the concentration of metal in the test substrate was substantially lower than the current national soil limit level guidelines of 140 ppm Cu and 300 ppm Zn (NZDH 1992). However, the outcome must be considered in relation to the bioavailability of copper and zinc in metal-spiked test soil substrate.

The artificial contamination of soils by spiking with metal salts has been clearly linked to increased soil acidification which increases the bioavailability of metals by increasing their solubility in soil pore water (van Straalen and Bergema 1995, Speir *et al.* 1999). Other factors which can elevate mobility include the oxidation-reduction potential of the metal salt and the decomposition of organic matter, as noted by Streit and Jaggy (1983). Chemical buffers such as $Ca(OH)_2$ are sometimes used to control acidification in metal spiked soils, however the acidification effect is by no means universal for all metal-salt amended soils (Liang and Tabatabai 1978, Bollag and Barabasz 1979). My results clearly demonstrated an acidification effect for both Cu and Zn added as chloride salts, which does not support the findings of either Liang and Tabatabai (1978), Bollag and Barabasz (1979), or Haanstra and Doelman (1991). My results do reflect the valid concern expressed by Speir *et al.* (1999) who attributed the inhibition of a number of biochemical activities to a soil acidification effect, rather than a direct metal effect.

However, it might also be argued that most published toxicity tests qualify the results by a demographic response, in a given substrate, at a specific concentration, pH and % soil organic matter (Will and Suter 1994). Given that the comparability of toxicity test results is often hindered by methodological variability, it could be argued that the potentially confounding effect of enhanced bioavailability may actually be valuable. This is because it extends the variability of conditions eliciting a response which, when examined across a range of species, substrates and methodologies, enhances the confidence with which a guideline judgment can be made.

The experimental outcomes identify a clear linkage between metal concentration and the growth rate of an organism. Thus, the outcomes support the preliminary "scope for growth" effect presented by Maltby and Naylor (1990) for *Gammarus*. The reduction in growth exhibited by the cranefly larvae reinforces the value of the organism-level approach to studying the effects of stress on physiological processes advocated by Maltby (1999). This is because it helps to better understand the stress tolerance of species and enables prediction of population-level effects which ultimately relate to biodiversity issues.

One unanswered question that arises from the outcomes is the possibility of suppression or stimulus of gut fauna by ingested metals, including parasites sensitive to specific metals. Such and effect was suggested by Bengtsson and Gunnarson (1985) when examining the influence of metals on reproduction in the collembolan *Onychiurus armatus*. Whilst larval growth was suppressed at 60 ppm Cu, there was no significant difference in growth impairment between that concentration and the higher concentrations tested for Cu. Thus, it is possible growth may have

been suppressed by either external mechanisms (such as palatability or availability) or by internal mechanisms (gut flora and fauna). It is interesting to note that the acidity of the substrate was extremely low in the 120 and 150 ppm Cu treatments (pH 1.52 and 1.46 respectively), compared with the 60 ppm Cu pH of 4.65. However, it must also be noted that the larvae continued to gain some biomass, irrespective of Cu treatment rate. Suppression of the gut fauna could be a valuable area for further investigation of metal associated effects.

The experimental protocol was novel in that it allowed for a recovery period, in which larvae were returned to uncontaminated soils and growth, survival and WBMC parameters were subsequently compared. Perhaps the most consistent effect observed was the capacity to excrete ingested Cu and Zn, irrespective of previous exposure. The percentage change in WBMC was consistent for the Cu treatments but variable for the Zn treatments. Elimination capacity has not, to my knowledge, been examined for dipteran larvae and this outcome represents a new direction in quantifying the effects of chronic Cu and Zn responses and physiological processes. Other studies, such as that of van Gestel *et al.* (1983) have examined metal elimination capacity in the earthworm *Eisenia fetida*, whilst Timmermans and Walker (1989) reported WBMC concentrations before and after metamorphosis of chironomid larvae. Direct accounts of metal elimination efficiency in actively feeding larval stages are not available.

Extrapolation of the metal detoxification pathway models proposed by Hopkin *et al.* (1989) for the spider *Dysdera crocata* and the woodlouse *Porcellio scaber* to cranefly larvae suggest Zn is a Type B metal which enters digestive cells and resides as a precipitate on the cytoplasmic side of microvilli; Cu is a Type B metal which enters digestive cells via the cysteine-rich protein metallothionien and is deposited in association with suplhur as cellular granules. These pathways implicate the need for protein-rich material to aid the detoxification process which, in the context of the scope for growth theory, implicates the need for appropriate allocation of energy reserves to inactivate potentially lethal metals. Importantly, Hopkin *et al.* (1989) noted the possibility of spillovers between these apparently segregated pathways when uptake sites on the cell membrane are saturated in heavily contaminated species. Such events could cause cellular disruption and limit the digestive process, further restricting growth parameters.

8. Species responses to a contaminated habitat: evidence at the cellular level

The subsequent histological examination of the cranefly larvae provided convincing evidence of a physiological response to Cu and Zn which was clearly attributable to a differential effect on growth. The most convincing support was found in the comparison between the fat bodies of

larvae from Cu and Zn treatments and control larvae. Ultrastructural differences in the midgut of treated larvae also point to physical damage to the gut tissues. However, it is not possible to attribute cause and effect directly to the bioavailable metal, as the extremely acidic test conditions may have been influential in damaging the epithelium and basal membranes.

Many other studies have successfully identified cellular alterations in response to metal contaminants. However, I am unaware of any other studies directly seeking histological evidence to support the scope for growth hypothesis. The histological results clearly demonstrated a differential effect, particularly in relation to the fat bodies, which supports the quantified difference in growth which was demonstrated in the previous chapter.

The histology also identified a methodological difficulty which can confound the use of WBMC as a useful metric. Because digestive contents were variably retained by the larvae, there are implications, not only for the future use of these larvae in the laboratory, but also for comparisons between the WBMC of laboratory specimens and the WBMC of wild populations. This is because WBMC has been used in conjunction with the background concentration of metals in soils to estimate bioavailability. A true measure of bioavailability in relation to WBMC may be dependent on effective gut elimination, for which it is necessary to understand and manipulate the processes driving gut evacuation.

The outcomes of the histological work are of relevance in the context of bioindicators, biodiversity and biosolids, because they demonstrate the chronic physiological effects of metals which accumulate in biosolid-amended habitats on an ecologically relevant bioindicator. The research has implications which relate to the value of cranefly larvae as bioindicator organisms. In view of the tolerance of the *Leptotarsus* sp. larvae to the extreme treatment conditions and, given that they continued to grow during exposure, the juvenile stage of this genus can be recommended as a hardy and resilient laboratory tool.

C. LIMITATIONS OF THE RESEARCH

As the survey and experimental work for this research progressed, some limitations became apparent. These are as follows:

(i) The taxonomic inventories, although valuable in establishing baseline data for local biodiversity and fulfilling a well-defined gap in the entomological understanding of the invertebrate assemblage associated with mid Canterbury's *P. radiata* forests, were time consuming. These surveys enabled the effective evaluation of potential indicator species for

subsequent environmental evaluation, but the timeframe for the research did not enable experimentation and validation of these indicator species. For this reason, the effectiveness of the suggested species in defining possible ecological effects following repeated applications of biosolids is unknown. This limits the immediate value of the research to forest managers.

(ii) A second limitation was also related to time. Delays in the development and trialing of the purpose-built biosolids trailer spreader meant that there was only one seasonally-suitable opportunity to sample the invertebrate population. Furthermore, there was no opportunity to sample sites which had received more than one application. These areas remain open for further investigation.

(iii) There were a very limited number of suitable reference studies in relevant east coast locations available at the beginning of the research. It may have been beneficial to more closely review these studies to identify possible instances of species repetition across sites. Species or species-specific behaviours could have examined under controlled conditions to measure the biosolid-mediated responses, particularly with reference to behaviour and the physical effects of biosolids.

(iv) The use of functional analysis for soil and litter invertebrates can be criticized because trophic allocation is dependent on an understanding of species biology, which is often found wanting. Furthermore, the basis for a simple trophic classification may be suspect because many animal species have a mixed diet which may range from extreme generalist to highly selective. For these reasons, the functional analysis may represent a simplistic view of trophic allocation, particularly amongst the omnivorous, detrivorous and mycetophagous species.

(v) One of the problematic areas in ecological investigations is the decision to conduct robust and detailed experiments across a limited number of sites, or to use a greater number of less rigorously designed experiments across a large number of sites. The usual factors determining the choice are resources and the level of detail required. To help solve the dilemma, models are proposed and then validated at greater or lesser scales of resolution. The model relating species diversity (H') to the stage of development of a stand was a case in point. The model is limited because only four sites representing a gradient of age classes were used and the experimental design provided detailed information on a very localized scale. Therefore, its applicability beyond mid Canterbury remains questionable.

(vi) The final limitation was identified in relation to the chronic toxicity experiment. Although a preliminary experiment was undertaken to establish a baseline methodology, the entire experimental area requires greater attention to confounding factors such as soil acidification, as well as identification of possible conflicts in optimizing stain effectiveness. It was also clear that more statistically robust results could have been shown had more larvae been taken through the experimental process. Although more replicates is often desirable, it may not always, as was the case here, have been economically feasible because of the costs of analysis.

D. FUTURE RESEARCH DIRECTIONS

The survey and experimental work successfully delivered a number of outcomes of relevance to the introduced and indigenous species associated with *P. radiata* planted forests. However, the process of the research also identified several possible areas for future research. The suggested areas are as follows:

(i) Resource constraints and taxonomic expertise are often limiting factors for entomological surveys. Yet it is widely recognized that invertebrates are valuable indicators of ecological effects, such as nutrient enhancement or contamination. This thesis presented statistically sound suites of invertebrates typical of the mid Canterbury planted forests which are recommended for future ecological assessment.

(ii) The predictable architecture and management strategies employed in planted forests and the fact that each stand can be considered as a replicate presents an appealing system to investigate broader ecological questions related to species assemblage. For example, on a regional scale, planted forests represent a series of fragmented habitats. How do factors such as the degree of isolation or the shape or the age of a fragment affect the biotic community? Which ecosystem processes are most affected by fragmentation. Such investigations may provide useful models to explore more complex forest systems.

(iii) Biosolids represent a slow release fertilizer, which is unlikely to have a quantifiable impact on the arthropod community in a short time frame. Yet quantifiable impacts may have bee experienced by other elements of the planted forest biota, such as bacteria and fungi. Microbiological studies of biosolid-mediated responses are suggested, particularly with reference to the ectomycorrhizal fungi which have an intimate association with conifers. This could have relevance for tree establishment and growth.

(iv) The methodology used in the soil column experiment was under-explored and represents a promising methodology for future research. This is because the linkage between species diversity and what a species does in the environment is integral to justifying the management of sites for biodiversity. The theories underlying the functional niche of species in ecosystems have been the starting point for much of the research justifying the management of resources for biodiversity. If there are more species present, are ecological processes enhanced? To what level can the abundance of species be restricted, such that ecological processes are not limited? Which species are important, and what sort of diversity is being measured? The methodology is robust and the environmental parameters can be finely tuned.

(v) The physiological effects of metal contaminants have been a rich area of research for some decades. One of the most appealing and least conclusively addressed interpretations of the toxicity study was the possibility that ingested metals may have affected gut fauna. This has interesting and important implications for the success of individuals and populations where growth may be dependent on the adequate nutrition of these fauna. This could be one of the factors restricting the relative success of indigenous species in modified habitats where resources either fail or oversupply specific nutrients necessary for digestion of ingested materials.

E. CONCLUDING STATEMENT

Biosolids applications represent a novel disturbance event in the managed forest habitat. Mid Canterbury's planted forests are characterized by a very distinctive arthropod assemblage and it has been demonstrated that these forests do not, in their own right, want for diversity. In this thesis there is substantial evidence that a very diverse fauna, generally characterized by generalist species tolerant of human modification inhabit the forests. The data presented is a substantial and timely step forward in acknowledging the reservoir value of the planted forest for local biodiversity. The arthropod assemblage is generally predictable for the age class of the stand and diversity tends to increase logarithmically from the stage of establishment through to economic maturity. Although functional and species diversity (H') were not shown to be affected by biosolid applications up to 800 kg N/ha, there is evidence of quantifiable effects at the species level. For example, the novel bioindicator *Leptotarsus* spp. was shown to have a significant negative response to the physical effects of biosolid amendment at 400 kg N/ha. This bioindicator subsequently displayed significant negative demographic responses to Cu metal-salt amended soils, although they have the capacity to recover at some dose rates. In order to link this outcome to biosolids amendment in plantations, further work is required to determine the availability of Cu in biosolids to determine its availability and assess if the loading rates are within those from

which the larvae can recover. The research demonstrated linkage at the cellular level of changes to the gut ultrastructure following exposure to acidic, metal-contaminated substrates.

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APPENDICES

APPENDIX A:
RAINFALL AND TEMPERATURE DATA
FOR HORORATA WEATHER STATION

This table provides background weather data for the nearest weather station to the study site referred to in the text.

Table A1 Rainfall (mm) and temperature (°C) data for Hororata, the closest weather station to the experimental sites. (Source: New Zealand Climate Digest, National Institute of Water and Atmospheric Research (NIWA) Ltd.)

Month and Year	Rainfall		Temperature °C		
	Total (mm)	No. of days	Max	Min	Average
January 1999	65	12	24.6	11.0	17.8
February, 1999	54	5	24.4	9.2	16.8
March, 1999	89	10	22.5	9.6	16.1
April, 1999	61	17	16.3	5.2	10.8
May, 1999	17	6	17.0	2.9	10.0
June, 1999	84	13	13.1	-1.3	5.9
July, 1999	130	20	10.8	-0.5	5.2
August, 1999	49	15	12.6	0.3	6.5
September, 1999			15.6	2.2	8.9
October, 1999	75	13	18.0	6.2	12.1
November, 1999	117	16	18.0	7.0	12.5
December, 1999					
January, 2000	101	22	19.9	9.0	14.5
February, 1999	41	12	22.1	6.1	14.1
March, 2000	75	9	21.0	3.8	12.4
April, 2000	108	16	17.3	1.9	9.6
May, 2000	58	11	15.7		
June, 2000	41	15	13.6	1.4	7.5
July, 2000	25	12	12.7	0.3	6.5
August, 2000	216	14	12.0	0.4	6.2
September, 2000	59	16	14.8	3.2	9.0
October, 2000	41	13	18.4	4.3	11.4
November, 2000	79	14	16.7	2.9	9.8
December, 2000	44	10	24	9	16.5
January, 2001	69	15	22.4	8.2	15.3

APPENDIX B:
FIVE DIGIT IDENTIFIERS FOR INVERTEBRATES

This table provides the species names linked to the four-symbol identifiers used in DCA analysis in the text.

Table B1. The 6-digit identifier for the invertebrate taxon used in the DCA ordinations.

TAXONOMIC GROUP	Identifier	TAXONOMIC GROUP	Identifier
Neotrichozetes RTU1	NEOSP1	*Pentagonnica vitipennis*	PENVIT
GAMISIDAE	CAMSP1	*Adriopea* sp.	ADRSP1
PARASITIDAE	PARSP1	*Xyloteles costipennis*	XYLCOS
CUNAXIDAE	CUNSP1	*Pristoderus antarcticus*	PRISP1
Prostigmata RTU1	PROSP1	*Hylastes ater*	HYLATE
Nuncia sp	NUNSP1	Histeridae RTU1	HISSP1
Phalangium opilio	PALSP1	*Odontria varicolorata*	ODOVAR
PSEUDOSCORPIONIDEA	PSESP1	*Agrypnus variabilis*	AGRVAR
Anapidae RTU1	ANASP1	*Otiorhyncus ovatus*	OTIOVA
Clubionidae RTU1	CLUSP1	*Conoderus exsul*	CONEXS
Aparua kaituna	APAKAI	*Pycnomerus sophorae*	PYCSOP
Hemicloea rogenhoferi	HEMROG	*Epuraea* RTU1	EPUSP1
Dysdera crocata	DYSCRO	*Acrotrichus fascicularis*	ACCTAS
Anzacia gemmea	ANZGEM	Zopheridae RTU1	COLSP1
Linyphiidae RTU1	LINSP1	Zopheridae RTU2	COLSP2
Linyphiidae RTU2	LINSP2	Corticaridae RTU1	LATSP1
Linyphiidae RTU3	LINSP3	*Selenopalpus aciphyllae*	SELACI
Juvenile Linyphiidae	LINJUV	Staphylinidae RTU1	STASP1
Mynogleninae RTU1	MYNSP1	OMALIINAE	OMASP1
Erigone wiltonii	ERIWIL	STAPHYLININAE	STASP2
Diplocephalus cristatus	DIPCRI	COLLEMBOLA	COLLEM
Ostearius melanopygius	OSTMEL	*Icosidesmus varigatus*	ICOVAR
Microtenonyx subitaneus	MICSUB	*Schedotrigona* sp.	SCHSP1
Lepthyphantes tenuis	LEPTEN	*Ophyiulus pilosus*	OPHPIL
Lycosa hilaris	LYCHIL	*Cylindroiulus brittanicus*	CYLBRI
Lycosa n. sp.	LYCSP1	*Zelanion morbosus*	ZELSP1
Cambridgea sp.	CAMSP1	*Forficula auricularia*	FORAUR
Steatoda capensis	STECAP	*Leptotarsus dicroithorax*	LEPDIC
Achaeranea verruculata	ACHVER	*Leptotarsus zeylandiae*	LEPZEY
Trichananca fulgida	TRIFUL	*Myrmicinae RTU1*	FORSP1
Coleopteran larvae (non-predatory)	COLLAR	Coleopteran larvae (predatory)	CARLAR
Scarabaeidae RTU1	SCASP1	*Porcellio scaber*	PORSCA
Aphodius tasmaniae	ACRSP1	*Bobilla* sp..	BOBSP1
Coccinella undecimpunctata	COCUND	*Pleioplectron simplex*	PLESIM
Hypharpax australis	HYPAUS	*Conocephalus bilineata*	CONSP1
Laemostenous complanatus	LAECOM	*Hemiandrus* sp.	HEMSP1
Megadromus antarcticus	MEGANT		
Metaglymma moniliferum	METMON		

APPENDIX C:
REPORT ON A PRELIMINARY TRIAL TO DEVELOP AN EXPERIMENTAL PROTOCOL FOR A CHRONIC TOXICITY TEST USING LARVAL CRANEFLIES.

This report provided a protocol for the experimental work underlying Chapters Six and Seven.

A. INTRODUCTION

A preliminary metal uptake trial using cranefly larvae maintained in two soil types with differing contamination histories was conducted in the laboratory. The objectives of this trial were; (i) to establish a suitable protocol for a subsequent replicated trial; (ii) to develop a metal sequestration profile for the larvae and; (iii) to establish the minimum larval biomass necessary for AAS (atomic absorption spectrophotometry) analysis

B. MATERIALS AND METHODS

1. Soils

Two soil types were used:

(i) a Lismore stony silt loam (FORE) taken from a 25-year-old second rotation *P. radiata* planted forest located in mid-Canterbury. The forest soils had low background levels of metal

(ii) a sandy soil (BROM) from Paddock 17 at the Christchurch Wastewater Treatment Plant, Bromley. The locality of the soil was estuarine, however it was difficult to designate a specific soil type as the Bromley paddocks had received extensive waste fill, sewage sludge and industrial waste during the past 30 years.

Soil samples were taken from 5 plots (2 m apart) along a 10 m transect at each of two sites. Ten litres of sieved soil (mesh size 0.5 cm) was taken from each site (FORE: 0-10 cm depth, excluding surface needle litter; BROM: 0-10 cm depth, excluding turf), packed into large plastic bags and stored separately in two sealed bins in an air-conditioned (20°C) room. Five sub samples (approx. 50 gm each) were taken from each soil type, weighed and oven dried (105°C for 48 hours) to estimate the gravimetric moisture content. No adjustment was necessary to equilibrate moisture content.

2. Soil pH

Five 20 g replicates of air-dry soil were placed in a beaker containing 50 ml distilled water such that the ratio was (1: 2.5). The suspension was shaken by hand and the beaker then covered and left to stand overnight. A JENWAY 310 Microprocessor pH meter (Jenway Ltd., Essex) was standardized with buffer solutions of pH 4 and pH 7. The electrode was inserted into the suspension and pH was read to the nearest 0.01 unit. The electrode was rinsed in distilled water and excess water removed with tissue paper between readings. All measurements were made in a temperature-controlled room at 20°C.

3. Soil organic matter and carbon content

An indirect estimation of soil organic matter (SOM) and soil organic carbon (OrgC) was calculated from loss on ignition (LOI) values. There is good agreement between organic carbon content for Canterbury soils determined by chemical oxidation using the Walkley-Black procedure (Tiessen and Moir 1993) and by a direct combustion technique (Grewel *et al.* 1991). Five (approx. 10 g) air-dry replicates for each soil type were placed in porcelain crucibles and oven-dried at 105°C for 48 hours. Samples were cooled in a desiccator, weighed and then placed in a muffle furnace at 550°C for 4 hours. Samples were again cooled in a dessicator before re-weighing to calculate ash weight. Soil organic matter was estimated as the loss on ignition being the percentage weight loss that occurs when a soil is ignited in a furnace. The assumption that SOM contains 58% carbon was used to estimate soil organic carbon content.

4. Heavy metal concentrations in the experimental soils

Sub-samples of both soil types were initially oven dried at 105°C for 48 hours (McLaren and Cameron 1996). The concentrations of heavy metals (Cu, Zn, Cr, Ni, Pb) in FORE and BROM soils were determined by the Christchurch Wastewater Treatment Plant by atomic absorption spectrophotometry (AAS) (Hopkin 1989, Borovansky 1997). A standard method of nitric/perchloric acid digestion at 90°C for 2 hours was used, as this method dissolves all absorbed, chelated and otherwise bonded metals without attacking the crystalline metal content.

Results are given as the mean of two duplicates. Metal concentrations are given as parts per million dry weight.

5. Experimental Protocol

In mid July, 2000, approximately 180 cranefly larvae were extracted by hand from sieved soil (H horizon and mineral soil to 7 cm depth) from the *P. radiata* planted forest. Positive identification of live individuals to species level was not possible prior to experimentation, therefore larvae were visually sorted to include only individuals 7.5-8 mm in length. This minimized the probability of including individuals belonging to the comparatively larger summer emergent *L. albistigmus*, and the late summer emergents *L. tapleyi* and *L. zeylandiae* and also ensured most individuals were within the same developmental instar.

One hundred and twenty larvae were selected and placed into 4 plastic boxes (30 larvae per box) containing a sheet of filter paper which had been dampened with distilled water. Boxes containing larvae were maintained at 12°C and 80% relative humidity in a cabinet for 48 hours. This enabled evacuation of gut contents. Filter paper was changed twice daily. Gut clearance was necessary to eliminate ingested material, which could bias the chemical analysis of whole body metal content (WBMC) (Cain *et al.* 1995, Gräff *et al.* 1997).

There were six replicate experimental units for each of the soil types, BROM and FORE. Each unit consisted of a plastic, 450 ml screw-top jar containing 300 gm of either BROM or FORE soil. The lid of each container was perforated by a small central hole for aeration. Ten cranefly larvae were randomly allocated to each replicate. Containers were maintained for 4 weeks in a growth cabinet at 12°C and 80% relative humidity. The soil surface was lightly misted weekly with distilled water during this period.

At the end of the exposure period, the larvae from each of the six replicates for each soil type were bulked to form two samples, referred to as (i) BROM larvae or (ii) FORE larvae. Each sample was placed on damp filter paper for 48 hours in closed petri dishes in the growth cabinet under the same environmental conditions as described, to clear gut contents. Paper was changed twice daily. Larvae were then killed in boiling 8% formaldehyde (Hooper and Evans 1993) and oven dried at 40°C in covered petri dishes for 96 hours prior to AAS analysis.

6. Heavy metal concentrations in cranefly larvae

The concentrations of heavy metals (Cu, Zn, Cr, Ni, Pb) in cranefly larvae were determined by AAS at Bromley Wastewater Treatment Laboratory, Christchurch. The larvae were digested and

analyzed using a modified version of EPA 3050B methodology. This method consists of digestion of the sample with elevated pressure (and therefore temperature) in special vessels made of teflon, conducted inside a purpose-built microwave oven. The sample is weighed out into the vessel and 4 ml nitric acid and 1 ml hydrogen peroxide are added to the vessel, which is then capped then processed. The samples are cooled, made up to volume and digestates analyzed directly using graphite furnace AAS for elements below 100 ppm concentration and direct flame AAS for concentrations greater than 100 ppm.

The 120 larvae used in this preliminary trial provided 0.7 gm (oven dry weight) of material, from which duplicate samples (between 0.1 and 0.2 gm dry weight samples) for each soil treatment were analyzed by AAS. Whole body metal concentrations are given as the dry weight equivalent in parts per million (ppm). As samples were only duplicated, no standard deviation was calculated.

7. Analysis

The experiment was used only to provide a guideline methodology and baseline data of the whole body metal concentrations (WBMC) in the larvae and the background metal concentrations in both FORE and BROM soils, which were then compared with:

(i) New Zealand Department of Health (NZDH) limit values for metals in agricultural soils (Cameron *et al.* 1997) and;

(ii) United States Environmental Protection Agency (USEPA) toxicity screening benchmarks for metal limits in soils (Will and Suter 1994).

Accumulation factors (A_f) for Cu and Zn were calculated by comparing the element concentration in the larvae and their substrate. An $A_f > 1.0$ is indicative of element enrichment in invertebrates, whilst an $A_f < 1.0$ suggests the discrimination of elements from one trophic level to the next (Roth 1992).

C. RESULTS

1. Soil pH and %SOM

The BROM soil was 0.18 pH units more acidic than the FORE soil; BROM soil also had a higher organic matter content (Table C.1).

Table C.1 Soil pH, soil organic matter (%SOM) and soil organic carbon (%Org C) for FORE and BROM soils.

Soil Type	pH	% SOM	% Org C
BROM	4.83	15.73	9.12
FORE	5.01	12.09	7.01

2. Extractable metals in FORE and BROM soils

The concentrations of extractable metals from the two soil types differed substantially (Table C.2). Of the set of six metals analyzed, higher concentrations were extracted from the BROM soils than the FORE soils. The BROM soils exceeded NZDH limit levels for agricultural soils for cadmium (1.7 ppm) chromium (180 ppm), copper (200 ppm), nickel (27 ppm) and zinc (500ppm). By contrast, metal concentrations in the FORE soils were all within guideline limits. The USEPA screening benchmarks for soils were higher than the NZDHA guidelines for cadmium, lead and nickel. The wide discrepancy in the guideline limit value for chromium and the screening benchmark relate to the valence of chromium present. The commonly encountered form, Cr III is benign in comparison with the unstable Cr IV form and the screening benchmark has taken into consideration the more toxic form of the element. Limit levels for this metal have been contentious for some time and are currently under review (Ross *et al.* 1981, Speir *et al.* 1995).

Table C.2 NZDH (New Zealand Department of Health) limit values for maximum permitted metal concentrations in agricultural soils, metal concentration in BROM and FORE soils, whole body metal concentration (WBMC) in larvae exposed to BROM and FORE soils, and USEPA (United States Environmental Protection Agency) screening benchmarks for soils derived for significant effects on earthworms. All metal concentration values expressed as parts per million (ppm). Accumulation factors (A_f) are given as the ratio between the concentration of the metal in the soil and the WBMC of that metal in the larvae.

Metal	BROM soil	BROM larvae WBMC	BROM larvae (A_f)	NZDH limit values for agricultural soils	USEPA soil benchmarks[#]
Cd	4.7	1.8	0.38	3	20
Cr[X]	780	93	0.12	600	0.4
Cu	340	380	1.11	140	50
Pb	194	42	0.21	300	500
Ni	62	26	0.42	35	70
Zn	800	490	0.61	300	200
Metal	**FORE soil[a]**	**FORE larvae WBMC**	**FORE larvae (A_f)**	**NZDH limit values for agricultural soils**	**USEPA[#] soil benchmarks**
Cd	0.5	0.17	0.34	3	20
Cr[X]	15	8.5	0.56	600	0.4
Cu	6	39	6.5	140	50
Pb	21	8.5	0.4	300	500
Ni	19	5.2	0.27	35	70
Zn	53	12	0.22	300	200

*(McLaughlin *et al.* 2000)

[a] Determined by AAS at Bromley Wastewater Treatment Laboratory, Christchurch.

[#] (Will and Suter 1994)

[X] Limit values for Cr are currently under review

3. WBMC of larvae

The WBMC of larvae from the BROM soils was higher than that of the larvae from the FORE soils for all metals tested (Table C.2). As there was more metal in the BROM soils, greater uptake was expected. The larvae accumulated the metals, Cd, Ni and Zn, from the BROM soil at a higher ratio than the larvae from the FORE soils. The larvae in the BROM soils accumulated a lower ratio of Cu (1.11) than the larvae in the FORE soils (6.5). The larvae in the BROM soils accumulated a higher ratio of Zn (0.61) than the larvae in the FORE soils (0.22). The A_f gives a better indication of bioavailability than either WBMC or soil metal concentration individually, as it indicates the percentage of the metal present that is expected to be taken up. During the 4-week period of the experiment, larval survival was 100%. There was sufficient dry biomass for AAS analysis of samples in duplicate. Each duplicate was the bulked biomass of 30 larvae.

D. DISCUSSION

The research objectives met

This preliminary study met the two research objectives which were to; (i) develop a metal sequestration profile for the cranefly larvae maintained in the two soil types, BROM and FORE, and; (ii) establish that 120 larvae were required to provide sufficient dry biomass (between 0.1 and 0.2 mg dry weight per sample) for AAS analysis in duplicate.

Differential sequestration relates to metal bioavailability

The metal sequestration profile highlighted the capacity of the cranefly larvae to ingest and store metals. Sequestration from the BROM soil was higher than that from the FORE soil. There are a number of possible explanations for this. Firstly, the higher concentration of all meatals in the BROM soils meant that more were available for uptake. Secondly, the metals in the acidic BROM soil were probably more bioavailable than in the FORE soil (Speir *et al.* 1999). Thirdly, the microbial and fungal biomass which is grazed by the larvae may have bioaccumulated the metals in the BROM soil and preferential grazing of hyphae or fruiting bodies may at least partially account for elevated metal concentrations in the larvae. Soil fungi vary not only in their capacity to accumulate and immobilize metals, but also in the specific sites of accumulation within the fungal structures (Bowen *et al.* 1974, Turnau *et al.* 1998, Colpaert *et al.* 2000). Although nothing is known of the microbial biota associated with the BROM soils (M. Gilson, pers.com.), it is interesting to speculate on the presence of a soil microbe community tolerant of high metal loads in that locality.

Metal bioavailability is known to depend on several external factors, including soil pH, organic matter, the sorption and solubility behaviour of soils and the presence of soluble soil-borne substances. The internal bioavaialibility of metals may be influenced by cellular pathways, which inhibit or facilitate the movement of ions into the cells (Streit and Jaggy 1983, Peintner and Moser 1996, Welp and Bruemmer 1997, Rieuwerts *et al.* 1998, Turnau *et al.* 1998, Leita *et al.* 1999, Sayer *et al.* 1999).

Uptake pathways in larval craneflies

Gut analysis of the tipulid larvae used in this study suggested a polyphagic/geophagic trophic status in which both soil matter and fungi are ingested (pers. observation). The probable pathways of metal uptake include: (i) ingestion of soluble metal ions in the soil water; (ii) ingestion of metals adhered to soil particles and organic matter; and (iii) ingestion of metals previously accumulated by the fungal biomass in the soil. A significant uptake pathway via the epidermis is unlikely (as compared with the earthworm), as protection is possibly gained from the tough, cylindrical envelope encasing the larval body, which gives them their common name "leatherjacket".

Accumulation factors

Comparisons between BROM and FORE soils of the level of element enrichment or discrimination enabled predictions of the probability of sequential transfer of contaminants to successive trophic levels. Accumulation factors (A_f) are typically used to explain the relationship between an organism and its food source (Roth 1992). The larvae from both BROM and FORE soils had an $A_f > 1$ for Cu, suggesting element enrichment for Cu was occurring. The A_f of 0.61 for Zn for larvae from the BROM soils was almost 3 times that of the larvae from the FORE soils, however both were < 1, suggesting element discrimination was occurring for Zn. Both Cu and Zn are essential trace elements, although Cu is also toxic. Element enrichment typically occurs where there is a physiological demand for a trace metal in invertebrate metabolism, in excess of the normal supply via food resources. The pattern observed underlines the sensitivity of the larvae to changed metal concentrations, enhancing their value as indicators of effect.

Relating outcomes to guidelines

Of the six metals tested by AAS, both Cu and Zn were accumulated from BROM soil at levels far exceeding the NZDH soil metal limit guidelines (NZDH 1992). The WBMC of CU and Zn in the BROM larvae exceeded the screening benchmarks proposed by the USEPA for these metals (Will and Suter 1994).

Growth and survival

Larval growth and survival were not directly quantified during the preliminary experiment. Although the experimental protocol was not seen to have an effect on survival (as no mortality was observed) within the 4-week experimental timeframe, it was possible that hidden impacts of soil metal concentration on larval biomass and scope for growth may have occurred (Donker 1992). A measure of treatment effects on both survival and biomass were expected to provide a more precise guide to the demographic and physiological tolerances of the larvae to contamination at specified levels of concentration by specific metals.

Cellular-level sites of sequestration and effects on tissue morphology

Metal sequestration in the Diptera is typically associated with the cells of the mid gut (Filshie *et al.* 1971, Sohal *et al.* 1977, Hopkin 1990) and general pathways for uptake and detoxification have been proposed (Hopkin 1990). These findings provide a platform to further investigate the effect of metal contaminants on tissue morphology in relation to demographic parameters. This enables effects at the cellular level to be quantified in relation to observed effects at higher scales, such as growth. The "scope for growth" theory is of particular interest, because it can help explain the variable sensitivities of organisms and life stages to a contaminated habitat (Maltby and Naylor 1990).

Evidence for residual effects following chronic exposure

As a general rule, toxicology experiments follow a pattern of exposure at a specific range of levels, for a set period of time, yet rarely consider the capacity of the test organism to regain comparative fitness. Equilibrium may be reached by detoxifying the ingested metals and either storing them in an inactivated state or progressively shedding them in granule form (van Gestel *et al.* 1993). The capacity for metal shedding during the larval period has not, to my knowledge, been previously documented for the Diptera.

Conclusions and further research

The larval cranefly actively ingests and expels surplus accumulated metals. The effect of this process, particularly detoxification, on the "scope for growth" of the larvae is unknown. As these larvae appear amenable to laboratory manipulations, further research using these novel bioindicators may be valuable in linking responses at different scales.

APPENDIX D:
RESEARCH PROPOSAL MANUSCRIPT

This manuscript was developed in the early stages of the research reported in this thesis. Material from the manuscript was presented at the "Pollution Effects-Biomarkers in Environmental Toxicology Conference" held in Christchurch, New Zealand, 14-16th July, 1999. This paper was subsequently published in 2000 in the Australasian Journal of Ecotoxicology **6:** 31-34.

The manuscript provides a short overview of the use of nutrient-enhancement of plantation forests with biosolids. Proposed methodologies for quantifying invertebrate biodiversity are outlined. The larval cranefly *Leptotarsus* spp. as a potential and novel bioindicator of effect is introduced. The paper establishes the hierarchical framework subsequently adopted in the thesis.

CHRONIC HEAVY METAL CONTAMINATION IN INTENSIVELY MANAGED *PINUS RADIATA* PLANTATIONS: A PRELIMINARY REPORT

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Received, 14/07/99; accepted, 28/02/00.

ABSTRACT

Heavy metal accumulation in forest floor soil and litter is the key factor limiting the sustainability of biosolids (municipal sewage sludge) application in intensively managed *Pinus radiata* plantations. In New Zealand, these exotic plantations provide habitat for a distinctive, yet limited forest floor fauna with a poorly known ecology. The value of this fauna in decomposition processes and nutrient recycling is broadly acknowledged. This work in progress examines invertebrate biodiversity and the utility of representative invertebrate groups as bioindicators in a production forest habitat, where biosolids application is proposed as part of a sustainable and intensive management plan. During a year-long preliminary characterisation study of the fauna in Mid Canterbury pine plantations, large populations of the fungivorous larval cranefly *Leptotarsus dichroithorax* (Diptera: Tipulidae) were found at the interface of the organic and mineral horizons. These larvae are being assessed for their efficacy as bioindicators of chronic, sub-lethal heavy metal contamination. Estimates of probable exposure, dose-effect and dose-response relationships are being quantified using field cage experiments in parallel with laboratory based uptake trials. Accumulation patterns and physiological responses are being determined from histopathological examination. Growth, morphology and fecundity are also examined. The expected outcome will assist both forest and local council management to better estimate the sustainability of biosolid redistribution in the production estate.

Key words: *Pinus radiata*; heavy metals; biodiversity; biosolids; invertebrate bioindicators.

BACKGROUND

International agreements and national standards recognise the importance of conserving biodiversity as part of sustainable forest management. For example, the Montreal Process (Lammerts van Bueren and Blom 1997) emphasises the need to maintain fundamental ecological processes and monitor the ecological continuity of functionally important species in the forest habitat. The draft New Zealand Biodiversity Strategy (Anon. 1998) aims to protect biological diversity through the promotion of sustainable management in productive ecosystems.

The enhancement of nutrient-deficient forest soils through the addition of nitrogen-rich biosolids is one means of increasing fibre productivity, while providing a location for the redistribution of municipal waste (Henry *et al.* 1994). The sustainability of such a process should therefore take into account the ability of the forest ecosystem to absorb and benefit from the nutrients, without encountering irreversibly detrimental effects from the heavy metal contaminants in the biosolids (Sauerbeck 1987).

1. Invertebrate diversity under *Pinus radiata* D. Don

Little is known of the diversity, functional status or taxonomy of ground invertebrates in New Zealand's exotic *Pinus radiata* forests. Yet the handful of surveys conducted in South Island plantations indicate a distinctive, if limited, assemblage (McColl 1974, Johns *et al.* 1980). A year-long characterisation of the forest floor fauna in two pine stands at different stages of rotation (5 y and 25 y), growing on similar soils, was undertaken in the Dunsandel district, Mid Canterbury, between December 1998 and January 2000. Invertebrates were sampled seasonally, by pitfall and pan trapping, in conjunction with litter and soil extraction using Berlese funnels and a modified Baerman apparatus. The adult forms from five Orders (Arachnida, Coleoptera, Orthoptera, Dermaptera and

Nematoda) and the immature forms of Diptera and Coleoptera were targeted as they represent some of the most abundant arthropod fauna in the pine forest habitat. All invertebrates were sorted to species (or RecognisableTaxonomic Unit where taxonomic status was unclear) and allocated to a functional group. At both sites, species richness, abundance, evenness and association are being examined in conjunction with physical and chemical parameters; ordination techniques (PC-ORD) (McCune and Mefford 1995) will be used to describe patterns in community assemblage.

2. Nutrient input and heavy metal contamination

Under the Resource Management Act (RMA), 1991 (Williams 1997), a consent granted to the Christchurch City Council (CCC) in 1999 permits applications of biosolids at a rate of 400 kg N/ha on selected *P.radiata* plantations. Conservative estimates suggest the project will be sustainable for 30 years, before cumulative metal loads will have reached maximum guideline levels (USEPA) for agricultural land (Woodward-Clyde 1996). Preliminary biosolid application trials in two Canterbury pine forests convincingly demonstrated the strong retention of heavy metals in the top 20 cm of the litter/soil horizons (McLaren *et al.* 1993). Arthropod biomass is highest in these horizons, especially in the presence of organic material (McColl 1974). The CCC is required to monitor heavy metal accumulation in the soil and litter as an integral part of the RMA consent, however this fails to identify either the bioavailability of metals, or the influence of nutrient enrichment on the litter/soil community. The separation of cause and effect at the community level may be confounded in such ecosystems (Yeates 1995, Larsen *et al.* 1996). This study will utilise baseline invertebrate biomass and abundance data obtained during site characterisation to estimate population fluctuations following biosolid applications. Atomic Absorption Spectrophotometry will be used to examine the whole body metal content of representative species. Metal contamination in this habitat is expected to be chronic, yet cumulative, over several years and may be below detectable limits in the early stages of treatment.

3. Larval Leptotarsus spp as bioindicators

Metal uptake, accumulation and toxicity studies using terrestrial arthropods (Dallinger and Rainbow 1991) and the acknowledged variability of responses within and between species (eg. Roth 1992), have led to a wide range of invertebrates being assessed as suitable indicator species. Maltby (1999) advocated an individual-level approach using an ecologically relevant species to provide both general and stress indicators, which can then be correlated with community level responses. For example, earthworms, a cosmopolitan group, are commonly used in routine ecotoxicological studies, largely due to their size, ease of management and functional status (Spurgeon and Hopkin 1999; Weeks and Svendsen 1996). Although earthworms are absent from Canterbury's pine plantations, larval Tipulidae may be a suitable substitute. Juvenile Tipulidae are present in abundance and contribute substantially to arthropod biomass (>50 individuals/ m^2 in mature forests). They do not move more than a few centimetres from the site of oviposition (Johns PM, unpublished) and they have a close functional relationship with soil fungi (Skerman 1953). Unlike some families of Tipulidae (Wiegers *et al.* 1992), *Leptotarsus* spp are relatively easy to raise under laboratory conditions. Mycorrhizal fungal feeding dipterous larvae have occasionally been used in metal accumulation studies (Lodenius 1981). Fungi are known to possess a variety of internal mechanisms for the uptake and inactivation of potentially toxic elements (reviewed by Lepp 1992), yet few attempts have been made to investigate the potential ecological significance in terms of elemental cycling or food chain contamination. The tipulid larvae are being used in an individual-level approach to examine their potential as bioindicators of habitat health and chronic heavy metal stress. Development parameters and histopathological responses will be quantified, where appropriate, following nutrient enrichment treatments and metal uptake trials. One metal of especial interest is chromium. Although trivalent chromium is generally accepted as being relatively benign in comparison with the less common and transient hexavalent chromium (Speir *et al.* 1995), there is uncertainty about the seldom addressed sub-lethal effects of chromium species on terrestrial invertebrates (Bartlett and James 1988).

4. Species description

The New Zealand tipulid fauna is represented by some 520 endemic species (Skerman, 1953). At least two species, *Leptotarsus zeylandiae* Alex. 1920 and *Leptotarsus dichroithorax* Alex. 1920 are common in soils, open ground and tussock scrubland (Johns PM., unpublished). Adults of both species have been captured in pantraps in the *Pinus radiata* habitat. Larval stages are found at the interface of the humic and soil horizons. The larvae are imperfectly known, although the pupal stage of *L. dichroithorax* was described by Rogers (1927) and the morphology of the larval stage headparts examined by Skerman (1953). It is not uncommon to see mass copulation occurring almost immediately after emergence, after which the female quickly oviposits then dies. Species dispersal may occur when pairs *in copulo* fly to new locations

(Johns PM, unpublished). The superficial similarity of the leathery abdomen and their shy habit of retracting the head capsule when disturbed can complicate the positive identification of live specimens. The larval and pupal stages of the as yet unidentified tipulid will be described as part of this work.

SUMMARY

This research utilises a tiered approach to ecotoxicology. Although environmental impacts are best quantified at the population level, selection acts at the level of the individual. Understanding the physiological responses of individuals, such as *Leptotarsus* spp, to habitat contamination, enables predictions to be made for consequences at higher levels of organisation. As nutrient enrichment can alter existing decomposer community assemblages, an estimate of pre-treatment community structure is a necessary precursor for the comparison of post-treatment arthropod abundance and species richness in a biosolid-amended forest habitat. From the community level to the individual and cellular level, this study will provide a baseline for estimating the consequences of biosolid applications as part of an intensive forest management system.

ACKNOWLEDGEMENTS

This work is supported by the University of Canterbury T.W. Adams Scholarship in Forestry, a *Forest Research* NSOF Scholarship, Selwyn Plantation Board and Christchurch City Council.

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